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# CONTENTS OF VOLUME 27

FEBRUARY, 1941, NUMBER 1.

D. H. WENRICH, Portrait .....	Frontispiece
Wenrich, D. H. THE MORPHOLOGY OF SOME PROTOZOAN PARASITES IN RELATION TO MICROTECHNIQUE .....	1
Madsen, Holger. THE OCCURRENCE OF HELMINTHS AND COCCIDIA IN PARTRIDGES AND PHEASANTS IN DENMARK .....	29
Beaver, Paul C. STUDIES ON THE LIFE HISTORY OF <i>Euparyphium melis</i> (TREMATODA: ECHINOSTOMIDAE) .....	35
Culbertson, James T. TRYPANOSOMIASIS IN THE FLORIDA COTTON RAT, <i>Sigmodon hispidus littoralis</i> .....	45
McCoy, O. R., Vincent F. Downing and Stanley N. Van Voorhis. THE PENETRATION OF RADIOACTIVE PHOSPHORUS INTO ENCYSTED <i>Trichinella</i> LARVAE .....	53
Walton, A. C. AMPHIBIAN NEMATODES FROM THE GASPÉ PENINSULA AND VICINITY .....	59
McConnaughey, Bayard. TWO NEW MESOZOA FROM CALIFORNIA, <i>Dicyemene californica</i> AND <i>Dicyemene brevicephala</i> (DICYEMIDAE) .....	63
Young, Martin D. and Coyt Ham. THE INCIDENCE OF INTESTINAL PARASITES IN A SELECTED GROUP AT A MENTAL HOSPITAL .....	71
Culbertson, James T. NATURAL TRANSMISSION OF IMMUNITY AGAINST <i>Trypanosoma lewisi</i> FROM VACCINATED MOTHER RATS TO THEIR YOUNG... ..	75
Wharton, G. W. THE FUNCTION OF RESPIRATORY PIGMENTS OF CERTAIN TURTLE PARASITES .....	81
RESEARCH NOTES.	
Reardon, Lucy V. INCIDENCE OF <i>Endamoeba histolytica</i> AND INTESTINAL NEMATODES IN A GEORGIA STATE INSTITUTION .....	89
Stabler, Robert M. INTESTINAL PROTOZOA IN 106 PARASITOLOGY STUDENTS .....	90
Knutson, Herbert. THE OCCURRENCE OF LARVAE OF THE STABLE FLY, <i>Muscina stabulans</i> (ZETT.) IN LIVING NYMPHS OF THE GRASSHOPPER, <i>Xanthippus corallipes pantherinus</i> (Sc.) .....	90
Maldonado, José F. and W. A. Hoffman. <i>Tamerlania bragai</i> , A PARASITE OF PIGEONS IN PUERTO RICO .....	91
Hussey, Kathleen L. PARTIAL TWINNING IN A STYLET CERCARIA .....	92
Janer, José L. MIRACIDIAL TWINNING IN <i>Schistosoma mansoni</i> .....	93
Spurlock, G. M. A STOMACH TUBE FOR MICE .....	93
REVIEW .....	95

APRIL, 1941, NUMBER 2.

Leigh, W. Henry. VARIATION IN A NEW SPECIES OF CESTODE, <i>Raillietina (Skrjabinia) variabila</i> , FROM THE PRAIRIE CHICKEN IN ILLINOIS .....	97
Levin, Arthur J. RECOVERY OF <i>Trichinella spiralis</i> LARVAE IN EARLY STAGES OF INFECTION .....	107
Faust, Ernest Carroll, E. Perry Thomas and Jack Jones. DISCOVERY OF HUMAN HEARTWORM INFECTION IN NEW ORLEANS .....	115
Cort, W. W., Louis Olivier and D. B. McMullen. LARVAL TREMATODE INFECTION IN JUVENILES AND ADULTS OF <i>Physa parkeri</i> CURRIER .....	123
DeEds, Floyd and John O. Thomas. STUDIES ON PHENOTHIAZINE. IX. THE BILIARY EXCRETION AND ANTHELMINTIC ACTION OF THIONOL .....	143
Rodgers, L. Ormand. <i>Diplorchis scaphiopi</i> , A NEW POLYSTOMATID MONOGENEAN FLUKE FROM THE SPADEFOOT TOAD .....	153
Mizelle, John D. STUDIES ON MONOGENETIC TREMATODES. IV. <i>Anchoradiscus</i> , A NEW DACTYLOGYRID GENUS FROM THE BLUEGILL AND THE STUMP-KNOCKER SUNFISH .....	159

Cheatum, E. L. <i>Dendritobilharzia anatinarum</i> n. sp., A BLOOD FLUKE FROM THE MALLARD .....	165
Hill, William C. <i>Gryporhynchus tetrorchis</i> , A NEW DILEPIDID CESTODE FROM THE GREAT BLUE HERON .....	171
Chandler, Asa C. HELMINTHS OF MUSKRATS IN SOUTHEAST TEXAS .....	175
RESEARCH NOTES.	
Chandler, Asa C. TWO NEW TREMATODES FROM THE BONITO, <i>Sarda sarda</i> , IN THE GULF OF MEXICO .....	183
Chandler, Asa C. A NEW SPIRUCROID NEMATODE, <i>Habronema americanum</i> , FROM THE BROAD-WINGED HAWK, <i>Buteo platypterus</i> .....	184
McNeil, E. and W. R. Hinshaw. THE OCCURRENCE OF <i>Hexamita</i> ( <i>Octomitus</i> ) <i>columbae</i> IN PIGEONS IN CALIFORNIA .....	185
Cuckler, Ashton C. and Lawrence R. Penner. <i>Cercaria elongata</i> BRACKETT, 1940, FROM A NEW SNAIL HOST, <i>Menetus exacuus</i> (SAY), IN MINNESOTA .....	187
IN MEMORIAM. JOHN EARL GUBERLET (1887-1940) .....	188

## JUNE, 1941, NUMBER 3.

CHARLES WARDELL STILES (1867-1941) .....	195
Christensen, John F. THE OÖCYSTS OF COCCIDIA FROM DOMESTIC CATTLE IN ALABAMA (U. S. A.), WITH DESCRIPTIONS OF TWO NEW SPECIES .....	203
Larsh, John E., Jr. <i>Corallobothrium parvum</i> n. sp., A CESTODE FROM THE COMMON BULLHEAD, <i>Ameiurus nebulosus</i> LE SUEUR .....	221
Hobmaier, M. DESCRIPTION AND EXTRAMAMMALIAN LIFE OF <i>Crenosoma mephitidis</i> n. sp. (NEMATODA) IN SKUNKS .....	229
Hobmaier, M. EXTRAMAMMALIAN PHASE OF <i>Physaloptera maxillaris</i> MOLIN, 1860 (NEMATODA) .....	233
Hobmaier, M. EXTRAMAMMALIAN PHASE OF <i>Skrjabinogylus chitwoodorum</i> (NEMATODA) .....	237
Chandler, Asa C. THE SPECIFIC STATUS OF <i>Moniliformis</i> (ACANTHOCEPHALA) OF TEXAS RATS, AND A REVIEW OF THE SPECIES OF THIS GENUS IN THE WESTERN HEMISPHERE .....	241
Manwell, Reginald D. AVIAN TOXOPLASMOSES WITH INVASION OF THE ERYTHROCYTES .....	245
Van Cleave, Harley J. and Charles M. Vaughn. THE TREMATODE GENUS <i>Otodistomum</i> IN NORTH AMERICA .....	253
Wilson, Francis H. THE SLENDER LICE OF AMERICAN PIGEONS AND DOVES WITH DESCRIPTIONS OF TWO NEW SPECIES .....	259
Kates, K. C. OBSERVATIONS ON THE VIABILITY OF EGGS OF LUNGWORMS OF SWINE .....	265
REVIEWS .....	273
AMERICAN SOCIETY OF PARASITOLOGISTS.	
SIXTEENTH ANNUAL MEETING, PHILADELPHIA, PA., DECEMBER 30 AND 31, 1940, AND JANUARY 1, 1941 .....	276
CHANGES IN MEMBERSHIP ROSTER .....	278
REPORT OF THE COMMITTEE ON NOMENCLATURE CONCERNING <i>Trichuris</i> vs. <i>Trichocephalus</i> , AND THE STATUS OF <i>Diectophyma</i> , <i>Diectophyme</i> AND <i>Eustrongylus</i> .....	279
ANNOUNCEMENT OF PLACE OF SEVENTEENTH ANNUAL MEETING .....	282

## AUGUST, 1941, NUMBER 4.

Pratt, Ivan and G. D. Barton. THE EFFECTS OF FOUR SPECIES OF LARVAL TREMATODES UPON THE LIVER AND OVOTESTIS OF THE SNAIL, <i>Stagnicola emarginata angulata</i> (SOWERBY) .....	283
Meyer, Marvin C. THE REDISCOVERY TOGETHER WITH THE MORPHOLOGY OF THE LEECH, <i>Branchellion ravenelii</i> (GIRARD, 1850) .....	289

Stunkard, Horace W. STUDIES ON THE LIFE HISTORY OF THE ANOPLORHYNCHAL CESTODES OF HARES AND RABBITS .....	299
Salis, Herbert. STUDIES ON THE MORPHOLOGY OF THE <i>E. histolytica</i> -LIKE AMOEBAE FOUND IN MONKEYS .....	327
Olivier, Louis and W. W. Cort. <i>Cercaria douglasi</i> CORT, 1917 AND ITS RELATION TO THE CERCARIA OF <i>Cotylurus flabelliformis</i> (FAUST, 1917) .....	343
Beaver, Paul C. THE LIFE HISTORY OF <i>Echinochasmus donaldsoni</i> N. SP., A TREMATODE (ECHINOSTOMIDAE) FROM THE PIED-BILLED GREBE .....	347
Landsberg, J. W. and C. F. Lischer. ANTHELMINTIC STUDIES ON THE MONOETHERS OF THE DIHYDROXY BENZENES .....	357
RESEARCH NOTES.	
Roth, Hans. OCCURRENCE OF <i>Trichostrongylus colubriformis</i> IN THE GUINEA PIG .....	363
Rothschild, Miriam. NOTE ON LIFE HISTORIES OF THE GENUS <i>Paramonostomum</i> LÜHE, 1909 (TREMATODA: NOTOCOTYLIDAE) WITH SPECIAL REFERENCE TO THE EXCRETORY VESICLE .....	363
Coulston, Frederick. THERMAL DEATHPOINT OF THE ERYTHROCYTIC STAGES OF <i>Plasmodium circumflexum</i> .....	365
Moorman, Albert E. <i>Balantidium coli</i> AND PINWORM IN A CHIMPANZEE .....	366
REVIEW .....	367

## OCTOBER, 1941, NUMBER 5.

Ripple, Richard C. STUDIES ON THE GAPEWORM <i>Syngamus trachea</i> (MONTAGU, 1811) IN ROBINS AND CHICKENS .....	369
Leonard, A. Byron and Alice E. Leonard. THE INTESTINAL PHASE OF THE RESISTANCE OF RABBITS TO THE LARVAE OF <i>Taenia pisiformis</i> .....	375
Wetmore, Psyche W. BLOOD PARASITES OF BIRDS OF THE DISTRICT OF COLUMBIA AND PATUXENT RESEARCH REFUGE VICINITY .....	379
Hopkins, Sewell H. NEW GENERA AND SPECIES OF THE FAMILY MONORCHIDAE (TREMATODA), WITH A DISCUSSION OF THE EXCRETORY SYSTEM .....	395
Noble, Elmer R. ON DISTRIBUTION RELATIONSHIPS BETWEEN CALIFORNIA TIDE POOL FISHES AND THEIR MYXOSPORIDIAN (PROTOZOAN) PARASITES ..	409
Farr, Marion M. and George W. Luttermoser. COMPARATIVE EFFICIENCY OF ZINC SULFATE AND SUGAR SOLUTIONS FOR THE SIMULTANEOUS FLOTATION OF COCCIDIAL OOCYSTS AND HELMINTH EGGS .....	417
Davis, Gordon E. <i>Ornithodoros parkeri</i> COOLEY: OBSERVATIONS ON THE BIOLOGY OF THIS TICK .....	425
McIvor, Barbara Catherine. A NEW SPECIES OF <i>Ornithodoros</i> TICK FROM NEVADA (ACARINA: IXODOIDEA) .....	435
Cort, W. W., Louis Olivier and Sterling Brackett. THE RELATION OF PHYSID AND PLANORBIID SNAILS TO THE LIFE CYCLE OF THE STRIGEID TREMATODE, <i>Cotylurus flabelliformis</i> (FAUST, 1917) .....	437
Tetley, J. H. SPICULE LENGTH IN <i>Cooperia curticei</i> AS A MEASURE OF FAVORABLE INTESTINAL ENVIRONMENT FOR THIS INTESTINAL NEMATODE OF SHEEP	449
Tetley, J. H. <i>Haemonchus contortus</i> EGGS: COMPARISON OF THOSE IN UTERO WITH THOSE RECOVERED FROM FECES, AND A STATISTICAL METHOD FOR IDENTIFYING <i>H. contortus</i> EGGS IN MIXED INFECTIONS .....	453
RESEARCH NOTES.	
Chandler, Asa C. A CASE OF URINARY MYIASIS .....	465
Travis, Bernard V. EXAMINATIONS OF WILD ANIMALS FOR THE CATTLE TICK <i>Boophilus annulatus microplus</i> (CAN.) IN FLORIDA .....	465
Katz, Julius S. A COLLECTION OF OHIO TICKS AND THEIR HOSTS .....	467
Goodnight, Clarence J. <i>Pterodrilus alvicornus</i> IN VIRGINIA .....	468
IN MEMORIAM. ADOLPHO LUTZ (1855-1940) .....	469
AMERICAN SOCIETY OF PARASITOLOGISTS.	
PRELIMINARY ANNOUNCEMENT, 17TH ANNUAL MEETING, DALLAS, TEXAS, DEC. 29-31, 1941 .....	472

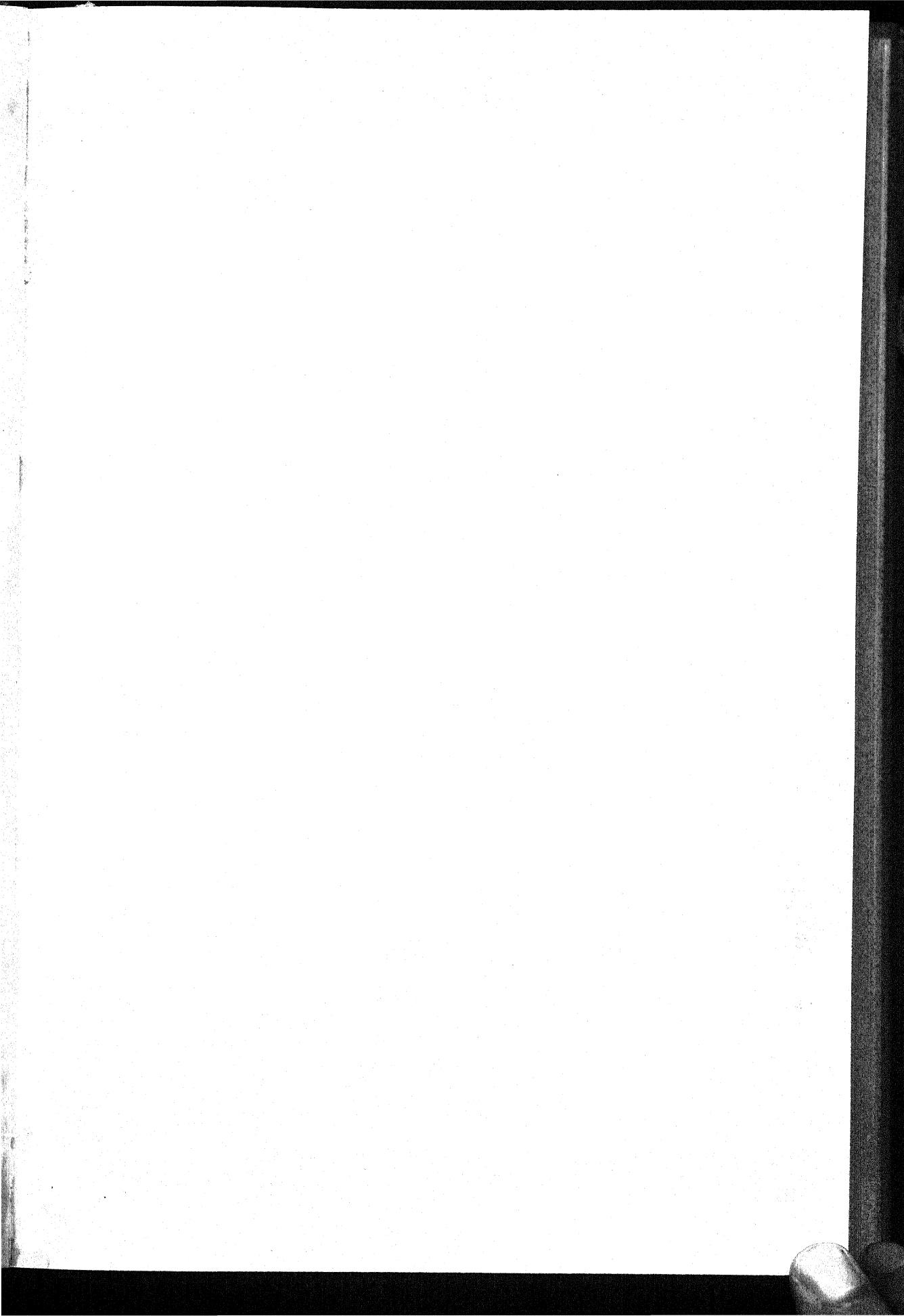


## DECEMBER, 1941, NUMBER 6.

Tetley, J. H. THE DIFFERENTIATION OF THE EGGS OF THE TRICHOSTRONGYLID SPECIES <i>Nematodirus filicollis</i> AND <i>N. spathiger</i> .....	473
Tetley, J. H. THE EGG-LAYING FUNCTION OF A NEMATODE AS SHOWN BY STUDY OF <i>Nematodirus</i> EGGS IN UTERO .....	481
Cort, W. W. and Louis Olivier. EARLY DEVELOPMENTAL STAGES OF STRIGEID TREMATODES IN THE FIRST INTERMEDIATE HOST .....	493
Lucker, John T. A REDESCRIPTION OF <i>Contracaecum multipapillatum</i> (VON DRASCHE, 1882) (NEMATODA: ANISAKINAE) .....	505
Hazard, F. O. THE ABSENCE OF OPALINIDS FROM THE ADULT GREEN FROG, <i>Rana clamitans</i> .....	513
Fischthal, Jacob H. and Leonard N. Allison. <i>Acolpenteron ureteroecetes</i> FISCHTHAL AND ALLISON, 1940, A MONOGENETIC TREMATODE FROM THE URETERS OF THE BLACK BASSES, WITH A REVISION OF THE FAMILY CALCEOSTOMATIDAE (GYRODACTYLOIDEA) .....	517
Feldman, Seymour I. STUDIES ON THE MORPHOLOGY AND BIOLOGY OF A PSILOSTOME FLUKE .....	525
Offutt, Edward P., Jr. and O. R. McCoy. THE "GOPHER," <i>Citellus richardsonii</i> (SABINE), AS AN EXPERIMENTAL HOST FOR <i>Trichinella spiralis</i> .....	535
RESEARCH NOTES.	
Hoff, C. Clayton. A CASE OF CORRELATION BETWEEN INFECTION OF SNAIL HOSTS WITH <i>Cryptocotyle lingua</i> AND THE HABITS OF GULLS .....	539
Ingram, William Marcus. THE HELMINTH FAUNA OF A RACCOON .....	539
Wohnus, J. Frederick and Dwight L. Ryerson. HEMATOZOA FROM CALIFORNIA BIRDS .....	540
Hardcastle, A. B. <i>Paragonimus</i> IN A CAT FROM NORTH CAROLINA .....	541
Stauber, Leslie A. THE POLYCLAD, <i>Hoploplana inquilina thaisana</i> PEARSE, 1938, FROM THE MANTLE CAVITY OF OYSTER DRILLS .....	541
Fujita, Tsunenobu. NEW NAMES FOR <i>Metabronema salvelini</i> FUJITA AND <i>Cystidicola minuta</i> FUJITA .....	542
Hegner, Robert and Redginal Hewitt. GENUS NAME <i>Martinezia</i> CHANGED TO <i>Martinezella</i> .....	542
INDEX FOR VOLUME 27, NUMBERS 1-6 .....	543
INDEX FOR DECEMBER, 1941, SUPPLEMENT .....	553

## DECEMBER SUPPLEMENT, 1941

AMERICAN SOCIETY OF PARASITOLOGISTS.	
PROGRAM, 17TH ANNUAL MEETING, DALLAS, TEXAS, DEC. 29-31, 1941 ....	1
AUTHOR INDEX .....	9
ABSTRACTS .....	10
OFFICERS .....	39
CONSTITUTION AND BY-LAWS .....	42





*D. H. Wenrich*

# The Journal of Parasitology

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## THE MORPHOLOGY OF SOME PROTOZOAN PARASITES IN RELATION TO MICROTECHNIQUE\*

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### INTRODUCTION

#### *The Importance of Technique*

To a considerable extent the diagnosis of parasitic protozoa, and certainly the study of their detailed morphology, is dependent upon the application of technical processes of fixation and staining, and the necessary accessory procedures. It might be well to emphasize that in the experience of my co-workers and myself, the diagnosis of intestinal protozoa is very much aided by the study of properly prepared fixed and stained smears. In our survey of college students (Wenrich, Stabler, and Arnett, 1935), we made a comparison of the relative efficiency of the examination of fresh smears in saline and iodine with that of the examination of fixed and stained slides. For 700 of the students, the stained slides furnished 11.2 per cent more positives than the fresh preparations. The advantages were different for different species. For example, 15.2 per cent more positives for *Endolimax nana*, 34.5 per cent more for *E. histolytica*, and 61.3 per cent more for *Dientamoeba* were detected through the study of stained slides alone than were seen on the fresh preparations alone. The tendency to get away from the study of prepared slides in making such surveys is to be deplored.

Because of the importance of the utilization of appropriate techniques in making permanent preparations, it seems worth while to consider some aspects of microtechnique in relation to the morphology of these parasites. In the following presentation consideration will be given primarily to intestinal protozoa.

For more than twenty years I have given much attention to intestinal protozoa and have therefore been forced to evaluate the relation between their apparent organization and the techniques employed in their preparation for study. It was soon realized that many protozoa present different appearances when treated in different ways and a certain amount of ex-

\* Address of the Retiring President, American Society of Parasitologists, December 31, 1940, Philadelphia.



perimentation with techniques was a natural result. Among the other conclusions from this study may be noted (1) that the same technique does not always produce the same result with the same species of parasite, because of the variability of the parasites themselves and the variability of the environments in which they are found; and (2) that different species often give very different results to the same technique because of inherent specific differences.

#### *Results Previously Published*

In successive publications from my laboratory, dealing with intestinal protozoa, some results of the employment of various techniques have been reported. I may refer to the work on *Trichomonas muris* (1921) in which it was noted that Schaudinn's fluid produced more shrinkage than Allen's modification of Bouin's; that cytoplasmic vacuoles showed stainable contents after fixation with sublimate-acetic but did not after fixation with Schaudinn's fluid; also that the parabasal body of this flagellate did not usually stain with Heidenhain's hematoxylin after fixation with Schaudinn's but did stain after Flemming's fluid and others containing chromic acid or osmic acid or both. Further, reference may be made to the studies of Freeman (1929) and Stabler (1932b) showing that various reagents were effective in producing "buds" on the cysts of *Entamoeba coli*, and that of Segal (1932) who reported similar results for *Endolimax nana*. In 1932 Stabler showed that when 15 to 20 per cent of acetic acid was added to the Schaudinn's fluid in which *Endolimax nana* was fixed, the peripheral layer of the nucleus in this species stained more distinctly with iron hematoxylin, while the endosome stained more faintly. In a joint note with Doctor Geiman (1933) it was pointed out one-half-strength Schaudinn's fluid gave as satisfactory a fixation of many intestinal protozoa as the full-strength mixture. The study on *Iodamoeba* (1937b) showed that higher concentrations of acetic acid in Schaudinn's fluid produced less stainability of the endosome of the cyst nuclei and that hemalum did not stain the endosome of cyst nuclei as intensely as Heidenhain's hematoxylin. In a paper on *Dientamoeba* (1937a) it was shown that the nuclei of this species usually fail to stain after fixation with Schaudinn's fluid unless a relatively high percentage of acetic acid is added, and that excellent stainability usually follows fixation with Bouin's or Hollande's fluids. The paper on nuclear structure and division in *Entamoeba muris* (1940) recorded among other things that the endosome does not stain with hemalum after fixation with Schaudinn's fluid and that there are two groups of chromosome-like bodies in the dividing nucleus one of which reacts to the Feulgen technique and the other does not, and finally reference may be made to the recent progress reports in the Yearbook of the American Philosophical

Society in which some of the results of more systematized experimentation techniques are briefly recorded (1938).

It will be both impossible and inappropriate to record all the variations in technique that have been followed during the past twenty years. Several thousand slides have been made and examined and over 130 different chemical agents or dilutions or combinations of them have been tried as fixatives. Not so much has been attempted with different staining methods. Certain of the more important results will be presented.

#### *Acknowledgments*

Since the material on which these studies have been based has been accumulated over a period of more than 20 years, I am indebted to many sources and a good many individuals for assistance. For financial assistance I am indebted to the Special Research Fund of the University of Pennsylvania, the Penrose Fund of the American Philosophical Society and a special research fund contributed by John Wyeth and Brother of Philadelphia. For assistance at various times in carrying out technical processes I am indebted to Doctor Quentin M. Geiman, Doctor R. M. Stabler, and especially to Doctor Sarah H. Stabler. For assistance in securing source material I am indebted to a good many persons but more especially to Doctors A. D. Waltz, R. M. Stabler, John H. Arnett, H. A. Shelanski, J. H. Clark, A. L. Luchi, and M. M. Rothman, and Mr. R. L. Brown. Mr. Brown also made the drawings for Figs. 71 and 72.

#### COMMENTS ON TECHNICAL PROCESSES

##### *Routine Procedures*

Fecal smears were made by diluting feces with modified Ringer's solution to make a thin paste which was spread out on a cover glass with the curved points of a small pair of forceps. The smear was then dropped smear-side down on the surface of the fixing fluid which was usually placed in a Petri dish. The smear was then turned over and placed with the smear-side up on the bottom of the dish. The first washing was generally in 50 per cent alcohol, after which the preparations were transferred to 70 per cent alcohol where they were kept until staining was undertaken. The graded alcohol series was usually 10, 30, 50, 70, 83, and 95 per cent, or the lowest three were 5, 15, and 30 per cent. From 95 per cent alcohol the preparations were cleared in a mixture of oil of thyme (3 or 4 parts) and oil of cloves (1 part) followed by xylol.

The Feulgen technique used was the standard one described in McClung's (1937) Handbook of Microscopical Technique, usually accompanied with Fast Green as a counter stain. In using iron hematoxylin, 4 per cent iron alum was used as the mordant, customarily for 24 hours or overnight, and 0.5 per cent aqueous hematoxylin as the stain, for an equal length of time.

*Temperature*

It is generally agreed that a warm reagent will act more rapidly than a cold one. It is a common practice to warm fixing solutions before use. Some years ago Miss Miriam Scott (now Mrs. A. M. Lucas) conducted temperature experiments for me with Schaudinn's fluid as the fixative and *Paramecium* as the experimental material. She found that fixing at 45 degrees C gave the most satisfactory results. Since that time I have fixed material at between 40 and 50 degrees or at room temperature. With the common intestinal protozoa, I have been unable to detect any significant difference as between fixation at the room or higher temperatures; hence, nearly all experimental fixations have been made at room temperature.

*Fixation Time*

It is customary to fix blocks of tissue for hours or days in many of the common fixing agents. It would seem obvious that thin smears would not require so much time. My experiments have shown that, in general, fixation for one minute produces as satisfactory a result as fixation for one to many hours. Often the shorter exposures produce better results than the longer ones.

*Heidenhain's Hematoxylin*

Iron alum hematoxylin is widely used and has the great merit of giving sharp differentiations of black-stained parts against backgrounds containing little or no stain. It is commonly employed for sectioned material and for smears. There are some obvious disadvantages in its use for smears. A relatively slight difference in thickness of a smear may make the difference between proper differentiation in the thinner areas and undestained black in the thicker areas, or, if the thicker areas are destained enough, the thinner ones become unstained. Bulky protozoa, such as many of the ciliates, do not usually give good results because of their too-great thickness. Some of the difficulties can be overcome by destaining with very weak iron alum, such as 1.0 or even 0.5 per cent, as recommended by Sharp (1914), or by destaining with a saturated aqueous solution of picric acid as suggested by Tuan (1930). Alcoholic solutions of picric acid, e.g., in 50 or 70 per cent, will destain much more rapidly, but the more rapid action may nullify the benefits of the slower action of the aqueous solution. I have found the aqueous picric destaining method advantageous for amoebae, ciliates and certain sporozoa. It has not been so successful for flagellates, because the differentiation of cytoplasmic structures is not so sharp as when destaining is done with 2 per cent iron alum.

The iron hematoxylin method as described in the books calls for mordanting for from 2 to 24 hours and staining for an equal length of time.

When quick results are not required it is often convenient to employ the 24-hour schedule. If haste is essential, the time can be reduced to 20 to 30 minutes at room temperature or even to 2 to 5 minutes if the temperature is raised to 35 or 40 degrees C as suggested by Craig and Faust (1937). I find these short-time treatments to be serviceable for diagnostic purposes, but they are often more capricious and less enduring than the results of the longer treatments.

#### *Other Stains*

I have experimented relatively little with different stains. The long time usually allowed for Heidenhain's hematoxylin makes it desirable to find a less time-consuming substitute for diagnostic purposes, at least. In many laboratories, Delafield's hematoxylin is the standard stain for tissue sections, and it has naturally been employed also for smears of intestinal protozoa. In my experience this stain is not very satisfactory for routine practice. Hemalum is quicker and, I believe, gives better results. It is not supposed to overstain, but if it does, it may be slowly destained with one per cent solution of potassium alum. After staining and washing the smear should be thoroughly alkalized either by a longer washing in tap water or a treatment with water containing a trace of ammonia. This stain is not advised for flagellates, since it does not stain cytoplasmic structures well. It may also fail to stain the endosomes of endozoic amoebae, hence its use must be accompanied by proper precautions in interpretation. Phosphotungstic acid hematoxylin has been recommended by some technicians, but it is less satisfactory than Heidenhain's. It may stain elements not stained by Heidenhain's and hence is valuable as a member of a series designed to bring out all possible structures.

#### *Feulgen's Nuclear Reaction*

It has often been surprising that a standard Feulgen procedure which results in typical coloring of nuclear chromatin in metazoan nuclei, may show a very weak coloring or none at all in the nuclei of protozoa. Nuclei of one stage in the life history of a protozoan may show a reaction while those of another stage do not. These diversities merit some comment.

Morris (1935) and Sassuchin (1935) reported that the nucleus of *Endamoeba blattae* showed no coloring by the Feulgen technique. Meglitsch (1939), however, found that while the nuclei of this amoeba failed to react in the non-dividing stage, when nuclear division took place, the chromosomes gave a positive reaction. In my own slides made from the intestines of cockroaches and treated by the Feulgen method, there were so few individuals of *E. blattae* that I could not determine the reaction for this species. However, the goodly number of specimens of *E. thomsoni* all showed the central granules of the nucleus giving a definite although not very strong Feulgen reaction.



For gregarines I obtained results similar to those reported by Reichenow (1928). In smears from the seminal vesicles of earthworms containing many acephaline gregarines fixed in alcoholic Bouin's fluid, and treated by the Feulgen method, the nuclei of the trophozoites failed to show a reaction although those of the spores and the dividing nuclei in the cysts all showed the typical coloring. On slides of *Cryptobia* (*Trypanoplasma*) from fresh water fish, the Feulgen technique produced a faint reaction in the nucleus but the kinetoplast (parabasal) was deeply colored. Jirovec (1927), Reichenow (1928), Cunha and Muniz (1928) and Sasuchin (1935) have reported similar results with hemoflagellates. Margolena (1932) and others have called attention to the fact that plant materials containing aldehydes, such as lignin, suberin, etc., give a positive nucleal reaction and on fecal smears it is common to see such materials showing the Feulgen coloration. It may therefore be stated (1) that nuclear chromatin does not always give a Feulgen reaction, and (2) that not everything that colors with Feulgen is chromatin. One might even question the frequently made statement that the Feulgen reaction is specific for thymo-nucleic acid.

For staining reactions in general, the dispersion factor is important. If a stainable material is finely divided and scattered among non-staining materials, the stain, if any, in these particles may be overlooked or masked by the associated substances. In the trophozoites of gregarines, for example, the failure of the nuclei to show a positive Feulgen coloration may be due to the great dispersion of the chromosome-forming materials in the relatively large nucleus which contains a large amount of non-chromosome-forming "chromatin." As a matter of fact, a close examination of some of these nuclei reveals the presence of very small scattered granules that show a faint Feulgen coloration. When this material is condensed into chromosomes at the time of nuclear division, the coloring is much more readily recognized.

In spite of the somewhat indefinite results obtained by the Feulgen method, it is, nevertheless, an important technical procedure and has proven to be very valuable in the interpretation of nuclear substances. Interpretation here, as always, needs to be made with due caution.

#### *Fixing Agents*

As soon as one attempts to experiment with different fixing agents one becomes aware that different solutions give different results. Here some comments will be made upon the use of some of the fixing agents used for fixing intestinal protozoa.

Schaudinn's sublimate alcohol, especially when a small amount of acetic acid is added, is probably the most generally useful fixing solution for intestinal protozoa. As a rule it provides for excellent nuclear dif-

ferentiation partially because it renders many cytoplasmic elements unstainable with the commonly employed stains. Fibrillar structures and basal bodies of flagellates and ciliates usually stain well but chondriosomes and some other cytoplasmic inclusions do not. This fixing agent usually produces considerable shrinkage, and for larger protozoa, like the large ciliates and gregarines, the distortion is often excessive. For these larger species, other fixing agents often give better results, such as Perenyi's fluid, Kleinenberg's fluid and Petrunkewitch's fluid for ciliates and alcoholic Bouin's for gregarines.

Bouin's fluid, especially the modification with saturated picric acid 75 parts, formol 15 parts and glacial acetic 10 parts, does tolerably well for the trophic stages of intestinal protozoa but it does not penetrate cysts well. It is excellent for *Dientamoeba*. Other standard fixing solutions, such as Hollande's, picromercuric, picroacetic, Kleinenberg's, Gilson's, Carnoy's, Destin's, Worcester's, and Flemming's fluids all have their usefulness for special purposes but do not possess the general adequacy that Schaudinn's fluid does.

The one-half strength Schaudinn's fluid recommended by Doctor Geiman and myself (1933) has proven to be very satisfactory for practically all the intestinal protozoa on which it has been tried. Even one-fourth strength has proven to be a satisfactory fixing agent for *Endolimax*, *Iodamoeba*, and *Entamoeba histolytica*. One-half strength Bouin's fluid has also proven to be as good as full strength when tested on *Entamoeba histolytica* and *Endolimax nana*.

When a saturated solution of mercuric chloride was diluted to three-fourths, two-thirds, one-half, one-third, one-fourth, and one-tenth strengths, the weaker solutions, that is, one-half or less, proved to be more satisfactory fixing agents than strong concentrations. Fifty per cent alcohol causes much less distortion of cytoplasm than stronger concentrations, although nuclear staining is much the same in all. Low concentrations of acetic acid, such as one, two, and five per cent proved to be more satisfactory fixing agents than stronger solutions. With picric acid, however, 50 per cent or higher percentages of a saturated solution gave better results than lower concentrations. One per cent hydrochloric acid was a fair fixing agent and better than stronger solutions.

The fact that for so many of these substances the weaker solutions were better fixing agents than stronger ones, leads to the belief that combinations of solutions much weaker than those commonly employed should be developed. They may be more satisfactory as fixing agents as well as being much more economical.

Formalin is not a satisfactory fixing agent for protozoa when nuclear details are of importance. Five per cent was not strong enough to coagulate fecal smears so that they adhered to the coverglasses. Stronger

solutions produced much distortion. Formalin does, however, definitely increase the reactivity of the peripheral layer of the nucleus of *Endolimax* for the Feulgen reaction as do also to a less extent, hydrochloric and nitric acids.

Approximately one hundred new combinations of mercuric chloride, alcohol, acetic acid, picric acid, chromic acid, with other substances added have been tried out as fixing agents, and while many of them have proven to be excellent fixing agents for protozoa, none of them appeared to be superior to Schaudinn's fluid, or its dilutions.

In the discussions which follow, the acetic acid used was glacial acetic. Hydrochloric, nitric, and sulfuric acids were Merck's C.P. diluted to the strength indicated. Unless otherwise stated, the stain referred to was Heidenhain's iron alum hematoxylin.

#### THE MORPHOLOGY OF CERTAIN SPECIES IN RELATION TO TECHNIQUE

##### *Trichomonas muris*

Reference has already been made to the fact that *T. muris* usually does not show a parabasal body when stained with Heidenhain's after fixation with Schaudinn's fluid. (In a few cases, however, I have seen the parabasal body of *T. augusta* when preparations were made in the same way.) After fixation with Flemming's fluid, or chrom-acetic, Heidenhain's may stain the parabasal. Cutler (1919) reported similar results with *Ditrichomonas termitis*. In 1926 Grassé discussed the literature up to that time and the results of studies by himself and his associates and believed that acetic acid is especially destructive to the parabasal body. Kirby (1931) reviewed the previous literature and showed that the parabasal body of trichomonad flagellates is stainable with Delafield's after fixation with Schaudinn's fluid, although not stainable with Heidenhain's. The difference in staining reaction of *T. muris* as between Delafield's and Heidenhain's stains after fixation with Schaudinn's fluid is even more striking than the statements reviewed above indicate. After staining with Heidenhain's, one sees that the blepharoplast, costa, chromatic margin of the undulating membrane, nuclear chromatin and various zones of cytoplasmic granules all stain intensely (Fig. 1). When stained with Delafield's, however, the costa, chromatic margin of the undulating membrane, and the parabasal body all stain deeply while the cytoplasmic granules are not stained at all (Fig. 2). After fixation in Schaudinn's without acetic, the parabasal did not stain nearly so well with Delafield's. When the same material was fixed with one per cent acetic alone and stained with Heidenhain's, only the endosome and the costa stained black; with Delafield's, the costa and peripheral layer of the nucleus stained very well but the parabasal was very poorly stained. When this material was fixed in 20 per cent acetic acid alone and stained with Heidenhain's, noth-

ing stained intensely and there was a wide vacant streak near the dorsal profile (Fig. 3). When Delafield's was applied to such a smear, the parabasal stained more intensely than any other structure (Fig. 4). It is therefore obvious that acetic acid does not destroy the parabasal body, at least in the strength of 20 per cent used alone as a fixing agent. When this same *T. muris* material was fixed in 2 per cent chromic acid containing 5 per cent of acetic acid and stained with Heidenhain's, the endosome of the nucleus and the costa were the only structures that stained intensely (Fig. 5), and the same was true for the slide stained with Delafield's. After Zenker's fluid as a fixative, Delafield's did not stain the parabasal body but did stain the central region of the axostyle, which does not ordinarily take a stain. It seems obvious, therefore, that the apparent morphology of *T. muris* varies widely depending upon the techniques to which it has been subjected.

#### *Giardia lamblia*

Although I have conducted a good many experiments with other kinds of trichomonad flagellates, limitations of time and space will not permit of their being discussed in this place. I should, however, like to refer to a few experiments with *Giardia lamblia*. Cysts of this species seem to be singularly uninfluenced by various fixing agents as judged by subsequent staining with iron hematoxylin. After most of the fixing solutions and their modifications that I have tried, there was little variation in the usual picture of a deep stain for the endosomes of the nucleus and for the various parts of the fibrillar apparatus. There were, however, differences in the amount of shrinkage inside the cyst membrane. For example, Schaudinn's without acetic gave rise to more intracystic shrinkage than when 2 to 15 per cent of acetic acid was added. In one batch of material fixation in chrom-acetic with a little copper oxide added produced practically no shrinkage of the protoplasm within the cyst membrane, in contrast to the usual fixing agents, but nothing in the cell stained with Heidenhain's stain. In another series fixation was made with Schaudinn's plus 5 per cent of acetic acid and with 5 and 25 per cent of acetic acid alone. For each of these the staining reaction with iron hematoxylin was practically the same, in the manner illustrated in Fig. 9. With the 25 per cent acetic fixation, the peripheral layer of the nucleus stained in some cysts. For each fixative, a smear was also stained in phosphotungstic acid hematoxylin. With this stain the peripheral layer against the nuclear membrane stained very well after Schaudinn's plus 5 per cent acetic, while the endosomes did not (Fig. 10). After the 25 per cent acetic acid fixation, however, the endosomes stained as well as the peripheral layer.

As reported by Lucas (1930) and Levitanskaia (1938), the endosomes of cyst nuclei nearly always give a strong positive reaction to the



Feulgen technique. They also stain strongly with hemalum, which, however, does not stain the fibrillar structures well.

Reference was made above to the differences in stainability with Heidenhain's on the same slide, when, of necessity, all phases of technique are the same. Such differences are illustrated in Figs. 6, 7, 8, and 9, each drawn from the same slide from regions destained about the same amount. In many cases, therefore, the variations for the same technique are greater than for different techniques. It is, therefore, necessary to exercise great caution in interpreting the results of different techniques.

#### *Iodamoeba bütschlii*

Since the genera of endozoic amoebae, and species also within the genus *Entamoeba*, are differentiated to a large extent by the structure of the nuclei, the effects of different fixing agents and stains on the appearance of nuclei has been given primary consideration in these experiments and receives attention in the following pages and the accompanying illustrations.

In a previous paper (1937b) attention was called to the fact that higher percentages (10 to 20 per cent) of acetic acid added to Schaudinn's fluid tended to reduce the stainability of the endosome in the cysts of *Iodamoeba bütschlii*. This tendency is subject to a considerable amount of variation. Fig. 11, for example, shows a cyst fixed in Schaudinn's plus 10 per cent acetic and stained with iron hematoxylin. The endosome does not look as solid as it ordinarily does if only 2 or 5 per cent acetic has been added. In many cysts on this same slide the endosomes were unstained. However, in one case no essential difference was noted in smears fixed in Schaudinn's fluid to which 5, 10, and 20 per cent of acetic acid had been added, while in another case all the endosomes were unstained when 20 per cent of acetic had been added to Schaudinn's. Schaudinn's plus 5 per cent acetic reduced to one-half and to one-quarter strength produced about the same result as the full strength solution.

When 1 per cent HCl, 5 per cent HNO<sub>3</sub>, 1 per cent urea and 5 per cent urea were each added to Schaudinn's fluid and tested separately, they all gave somewhat similar results so far as nuclear staining was concerned, the endosomes staining intensely black with Heidenhain's. There were differences in the effects on the cytoplasm.

When saturated mercuric chloride solution was used alone, cytoplasmic fixation was fair but the nucleus tended to stain a homogeneous black. About the same results were obtained with 75 and 50 per cent of a saturated solution. With 25 per cent and 10 per cent of saturation, however cytoplasmic fixation and nuclear differentiation were much better. When hemalum was used as the stain after 50 per cent of saturated mercuric chloride, the lightly stained endosome often contained a small deeply

stained granule (Fig. 12). Such a granule was noted in the paper on *Iodamoeba* already published, again after hemalum staining, but after Schaudinn's plus 10 and 20 per cent of acetic acid as the fixative. It is possible that this granule represents a "centriole" or division center.

Alcohol of a strength of 50 per cent produced less distortion and shrinkage of the cytoplasm of cysts and better differentiation of nuclei than 70 and 95 per cent. With hemalum the nuclear staining was about the same for all three strengths. When different strengths of acetic acid were used alone as fixing agents, they produced a good deal of cytoplasmic distortion, the amount increasing with increasing strengths. After 2 and 5 per cent the nuclei stained very well with black endosomes but after 10 per cent the staining ability of the endosomes decreased (Fig. 13), and after 20, 25, and 50 per cent acetic the nuclei remained unstained.

Saturated and 50 per cent of saturated picric acid fixed the cytoplasm very well, but the nuclei tended to overstain.

Bouin's fluid and several modifications of it were satisfactory fixation agents for trophic stages but produced much distortion of the cytoplasm and variability of staining in the nuclei of cysts. Alcoholic Bouin's was equally unsatisfactory for cysts and even produced cytoplasmic extrusions or "buds." Picromercuric, on the other hand, proved to be a very satisfactory fixing agent for *Iodamoeba* cysts.

#### *Endolimax nana*

*Endolimax nana*, as reported by Stabler (1932a), usually shows a loss of stainability of the endosome and an enhanced stainability of the peripheral layer of the nucleus with Heidenhain's staining after Schaudinn's with 15 to 20 per cent of acetic acid added. Figs. 14 and 15 show the trophic stage and cyst as they commonly appear after fixation with Schaudinn's plus 5 per cent of acetic acid added. Figs. 16 and 17 show the corresponding stages from the same fecal sample fixed with Schaudinn's plus 20 per cent acetic. The pronounced loss of stainability of the endosome is the typical condition. However, this reaction is subject to considerable variation. Figs. 18, 19, and 20 are all from a slide fixed in Schaudinn's with no acetic acid added. The great majority of the amoebae showed stained endosomes but some showed the endosomes unstained. Figs. 21, 22, and 23 are all from a slide from the same material fixed in Schaudinn's plus 20 per cent acetic. On this slide the majority showed stainless endosomes (Figs. 22, 23) but in some the endosomes stained deeply (Fig. 21). Addition of only 2 per cent of acetic acid has proved better than higher concentrations in some cases but not so good in some others. Schaudinn's plus 20 per cent acetic sometimes produces "buds" on cysts of *Endolimax*. Dilution of Schaudinn's did not produce any differences in stainability of the trophic stages.

Figs. 26, 27, and 28 are, respectively, from slides fixed in full strength, 50 per cent, and 25 per cent of the same. There were no noticeable differences in the results from the three strengths of this fixative.

When mercuric chloride was used alone in a saturated solution and in 75, 50, and 25 per cent of saturation, the weaker solutions proved to be better fixing agents than the stronger ones.

Acetic acid alone tends to restrict the stainability of the nuclei in both the trophic and cyst stages when stained in Heidenhain's. Even one per cent may have some effect in this direction and 20 per cent is followed by loss of stainability. Combined with other substances, acetic acid may have other effects. Figs. 29 to 38 all from the same source material, illustrate some results obtained by using mercuric chloride and alcohol with and without acetic acid, upon the stainability of the nuclei of trophic stages. After saturated mercuric (Fig. 29), the nuclei stain fairly well but not as darkly as when 5 per cent of acetic acid is added (Fig. 30). In this case, acetic added to the sublimate intensifies the stainability of the endosome. When 50 per cent of saturated mercuric chloride is used (Fig. 31), the endosome stains more intensely than when 5 per cent of acetic acid is added to it (Fig. 32). In the latter case, the tendency for the acetic to cause lighter staining of the endosome is effective against the weaker sublimate solution. When 50 per cent alcohol is used alone (Fig. 33), the endosome stains very black, but when 5 per cent acetic is added to it some of the stainability is lost (Fig. 34). Similar effects are seen for 70 per cent alcohol (Fig. 35) and the same with 5 per cent acetic added (Fig. 36). But when 95 per cent alcohol is used (Fig. 37), the endosome is apparently prevented from showing any loss upon the addition of acetic (Fig. 38). The effect of 5 per cent acetic by itself is shown in Figs. 39 and 40, where a somewhat lighter stain is obtained by differentiating with iron alum (Fig. 39) than with picric acid (Fig. 40).

Other acids, hydrochloric, nitric, and sulfuric in concentrations of 5 and 10 per cent of the C.P. solutions produced much distortion of protoplasm and variability in the staining capacity of the nuclei. Nitric acid was followed by better nuclear differentiation than either of the others.

Bouin's fluid sometimes produces shrinkage areas between the nuclei and surrounding cytoplasm in trophic stages (Fig. 25). It usually produces considerable shrinkage and distortion within cysts and irregularity of stainability of the nuclei. Picric acid in the one case when it was tried produced a fair result of both fixation and staining of trophic stages.

Segal (1932) has called attention to the fact that "buds" were produced on the cysts of *E. nana* more frequently when they were fixed in picromercuric than when fixed in Schaudinn's fluid and several other fixing agents. She also noted that different strains of *E. nana* had dif-

ferent tendencies in regard to the formation of "buds." The present study has confirmed the work of Segal. "Buds" were also produced to some extent by 25 and 50 per cent acetic acid.

Both hemalum and phosphotungstic acid hematoxylin, after most of the fixatives used, had a greater affinity for the peripheral layer of the nucleus than Heidenhain's hematoxylin.

*Dientamoeba fragilis*

Both Johnson (1935) and I (Wenrich, 1937a) have called attention to the fact that the nuclei of *Dientamoeba* do not usually stain well with Heidenhain's when fixed with Schaudinn's fluid unless the percentage of acetic added is as high as 15 or 20. This result has been confirmed many times but it has exceptions. For example, in some cases, when nuclei failed to stain after Schaudinn's plus 5 per cent acetic (Fig. 53), they stained very well when the same material was fixed in the same fixing solution reduced to one-half strength (Fig. 54); and in some other cases Schaudinn's plus 2 per cent acetic gave better staining than Schaudinn's plus 5 per cent, as did also this solution with 20 per cent acetic added. In still other cases the nuclei stain very well when the feces are fixed in Schaudinn's without acetic (Fig. 55). The feces represented by Fig. 55 was strongly acid to litmus paper at the time that the fixation was made. It is therefore suspected that various organic acids may enhance the stainability of the nucleus to Heidenhain's when sublimate-alcohol is the basic fixing substance.

The effect of acetic acid in combination with sublimate and alcohol is illustrated by Figs. 41-50; these figures are from the same slides that are represented in Figs. 29-38. Figs. 29 and 41 are from the same slide, as are Figs. 30 and 42, and so on through the series. Fig. 41 shows the nuclei barely discernible after fixation with saturated sublimate alone. When 5 per cent of acetic acid is added to this the nuclei stain very well (Fig. 42). With 50 per cent of saturated sublimate the nuclei are undifferentiated (Fig. 43), but with the addition of 5 per cent acetic they are well stained (Fig. 44). With 50 per cent alcohol the nuclei differentiate poorly (Fig. 45); but with the addition of 5 per cent acetic the nuclei stain distinctly. With 70 per cent alcohol (Fig. 47) the nuclei stain but the intranuclear granules are less distinct than when 5 per cent acetic is added (Fig. 48). The story is again similar for 95 per cent alcohol (Fig. 49) and the same strength with 5 per cent acetic added (Fig. 50). The effect of 5 per cent acetic alone is illustrated by Figs. 51 and 52. These are from the same slides as Figs. 39 and 40 and show no significant difference as between alum differentiation (Fig. 51) and picric acid destaining (Fig. 52).

This group of figures (Figs. 39 and 40; 51 and 52) shows the con-



trast between *Endolimax* and *Dientamoeba* in relation to acetic acid; this acid reduces the stainability of the central area of *E. nana*, but increases the stainability of the central area of *Dientamoeba*. Acetic acid of any strength appears to fix the nuclei of *Dientamoeba* so that they will stain more readily. Fig. 56 represents an individual from a slide fixed in full strength glacial acetic acid. The nuclei are fairly well stained but the granules of the central area are not too well differentiated.

It has been mentioned that Bouin's fluid, especially the B3 modification, is usually followed by excellent staining of the nuclei of *Dientamoeba*. Other fixing agents which give fairly satisfactory results are Hollande's, Kleinenberg's, Carnoy No. 2, Zenker's, and weak Flemming's. Hemalum is an excellent stain for *Dientamoeba*.

#### *Entamoeba histolytica*

As previously reported (1938), Schaudinn's fluid plus 5 per cent of acetic acid, or the same solution diluted with an equal quantity of distilled water, is about the most satisfactory fixing agent for *E. histolytica* that I have found thus far. Even one-fourth strength of this common fixative resulted in satisfactory fixation.

When different strengths of sublimate were used alone, the weaker solutions of 10, 25, and 50 per cent of a saturated solution gave better fixation than 75 or 100 per cent of saturated solution. Alcohol at 50 per cent gave better fixation and less distortion of the cytoplasm than 70 or 95 per cent, although nuclear staining was similar in all. In one case the use of 50 per cent resulted in the formation of a number of "buds" on cysts while none occurred when 95 per cent was used.

The stainability of the nuclei in Heidenhain's is much affected by acetic acid. If 20 per cent or more is added to Schaudinn's fluid or to other sublimate alcohol combinations, the nuclei tend to lose stainability, but the results vary with different cases. In one case where there were cysts of the small race of *E. histolytica*, the nuclei failed to stain when the smears were fixed in Schaudinn's without acetic. Nuclear stainability gradually increased with addition of 2, 5, and 10 per cent of acetic acid but diminished with the addition of 20 per cent.

When acetic acid was used alone even in strengths as low as 1 or 2 per cent, the stainability of the nuclei in Heidenhain's was diminished. One series of slides from the same material and stained with Heidenhain's is illustrated by Figs. 57 to 67. Figs. 57 and 58 show trophic and four-nucleated cysts from the same smear fixed in Schaudinn's fluid plus 5 per cent of acetic. The usual staining picture is revealed. When 5 per cent acetic alone was used as the fixative, the endosomes failed to stain while the peripheral layer of the nuclei retained its stainability (Figs. 59, 60). When 10 per cent acetic was used, the stainability was still more reduced



although retained better in the cysts than in the trophic stages (Figs. 61, 62). With 20 per cent acetic practically all stainability was removed from the nuclei (Figs. 63, 64). With 50 per cent acetic as the fixing agent, some stainability returned to the peripheral layer of the nuclei (Figs. 65, 66), but especially in the trophic stage (Fig. 65). Most of the cysts showed a relatively large shrinkage space between the cytoplasm and the cyst wall with this last fixative, and some of them showed relatively extensive "buds" protruding through breaks in the cyst membrane. These protoplasmic extrusions gave the appearance of having been produced explosively and the outer boundary was indefinite (Fig. 67). This type of "bud" is unlike those produced by other agents, such as Schaudinn's plus 5 per cent acetic (Fig. 68).

Bouin's fluid usually results in much intracystic distortion and irregularity of stain in the nuclei and in shrinkage spaces inside or outside the nuclei of trophic stages (Fig. 70). In many cases the endosomes fail to stain in the trophic stages after the use of B3 (Fig. 72). In this series of slides a very small endosome, perhaps the centriole, was usually visible when one per cent of urea was added to B3. Alcoholic Bouin's gives much the same results except that "buds" are more likely to be produced on the cysts. In one case 25 to 30 per cent of the cysts showed "buds" after the use of alcoholic Bouin when not more than 2 per cent were seen in the same material fixed with B3 and one-half strength B3. In this same material picromercuric produced between 45 and 50 per cent of "buds."

#### *Entamoeba coli*

Anyone who has examined any considerable number of series of slides showing *E. coli* cysts from different hosts is well aware of the variability seen when fixation is in Schaudinn's fluid and the stain is iron hematoxylin. In some cases there will be a high percentage of cysts that will be unstained; in others there may be a high percentage of cysts that are overstained; in still others there may be high percentages of both unstained and overstained cysts. In some cases there may be "buds" on some of the cysts and in other cases there will be none. Because of the variability of the organism itself, it is difficult to obtain consistent differences with different techniques.

Higher percentages of acetic acid added to Schaudinn's fluid have less tendency to diminish the stainability of the nuclei than with *E. histolytica*. Such loss of stainability that may occur affects the endosome more readily than the peripheral layer.

In one race which showed about 30 per cent "buds" on the cysts after fixation with Schaudinn's plus 5 per cent acetic, there were no "buds" when sublimate alone was used either in saturated or 50 or 25 per cent

of saturation. When the same material was fixed in 5, 10, 25, and 50 per cent of acetic acid, nuclear staining diminished from the 10 up to the 50 per cent acetic. No "buds" were seen after 5 and 10 per cent, but about 20 per cent of cysts showed "buds" after 25 per cent acetic and 90 per cent showed "buds" after the 50 per cent. In another case where 2 and 100 per cent glacial acetic were used as fixatives, there was scarcely any distortion after the 2 per cent and the nuclei stained very well, except that in the trophic stage the endosomes failed to stain in most of the nuclei. After the 100 per cent acetic there was usually a very pronounced shrinkage space between the cyst wall and the protoplasm on the cysts and in some the protoplasm was unstained and in others it was homogeneous black. In some cysts there were the same type of "buds" as seen in the other case cited above. In the trophic stages there was some stainable material in the periphery of the nuclei but none in the central area. The extrusions or "buds" produced by 50 and 100 per cent acetic were relatively enormous and gave the appearance of having been produced explosively. They usually failed to show any definite boundary layer making it difficult to recognize the contours. In this respect they were like the similar extrusions produced by 50 per cent acetic in *E. histolytica* (Fig. 57).

There seems to be fairly general agreement at the present time that Kofoid and Swezy's (1921) "*Councilmania lafleuri*" is only a variation of *E. coli* and that the "buds" and chromophile ridges described by these authors and considered by them to be diagnostic for "*C. lafleuri*" are produced by the agents commonly used in fixation. Freeman (1929) working in my laboratory, found that Yocum's picromercuric was highly effective in producing "ridges" and "buds." Stabler (1932b) reported that 95 per cent alcohol at 55 degrees C and picromercuric at 28 degrees were more effective in producing "buds" than the other agents tried. We can now add strong acetic acid to the list of effective "bud"-producing agents. The production of "buds" is a highly variable phenomenon, however, and these variations indicate either, (1) that certain races produce "buds" more readily than others or, (2) that "budding" occurs more readily in the same race under certain conditions presented by the surrounding feces than in other conditions. Probably a combination of these two possibilities is nearer the truth.

#### DIFFERENT SPECIFIC REACTIONS TO THE SAME TECHNIQUES

As previously pointed out the genera of endozoic amoebae have been differentiated from each other primarily on the basis of nuclear structure. A study of their reactions to varying techniques shows that these nuclei are not only different in organization but different in their chemical properties as reflected in different reactions to the same technique. An

attempt has here been made to demonstrate such differences by illustrating the results when the different species have been fixed the same way (in this case in Schaudinn's plus 5 per cent of acetic acid) but subjected to three different staining techniques.

On Plate IV Figs. 73-81 are from slides stained with Heidenhain's; Figs. 82-90 with hemalum and Figs. 91-99 with the Feulgen technique. All the figures of *E. coli*, *E. histolytica* and *Endolimax nana* are from the same source. Figs. 73-76, 79, and 80 are from the same slide; Figs. 82-85, 88 and 89 are from another slide from the same source; and Figs. 91-94, 97 and 98 are from still another slide of the same series. Figs. 77, 78, 86, and 87 are from one source, and Figs. 81 and 90 are from this same source. Only the material giving a deep staining reaction has been drawn in detail; this includes the chromatoids of *E. coli* and *E. histolytica*. In the drawings from the Feulgen slides the nuclear boundaries and other non-staining nuclear areas have been outlined only.

With iron hematoxylin the endosomes and the peripheral layer of granules stain deeply in *E. coli* and *E. histolytica*. In *Endolimax nana* the peripheral layer may or may not be readily discernible after this stain. With *Iodamoeba* the endosomes stain intensely as do also the periendosomal granules. In *Dientamoeba* the central nuclear area usually contains four or more deeply stained granules imbedded in a less deeply stained matrix.

With hemalum, the peripheral layer usually stains better than the endosomes in the species of *Entamoeba*. In *Iodamoeba* the periendosomal granules stain better than the endosome. In *Endolimax* the endosome normally stains well as does also the peripheral layer. In *Dientamoeba* the staining reaction is comparable to that with Heidenhain's. In *E. histolytica* the failure of the endosome to stain with hemalum is more often noted than with *E. coli*, but is not always observed in either species. In both species it will be noted that there is a certain amount of deeply stained material in the immediate vicinity of the endosome. This material is more voluminous in *E. coli*, making it more difficult to tell whether the endosome proper has been stained (Figs. 82, 83).

The contrast between the nuclear picture after the Feulgen technique and the other stains is very striking. In *E. coli* and *E. histolytica* only the material in the vicinity of the endosome shows the reaction (Figs. 91-94). In some cases where Fast Green was used as the counter stain, a small green granule could be seen amongst the Feulgen reacting granules, in the central nuclear area. In the cysts of *E. coli* the reacting material is often scattered (Fig. 92) as it often is also in the trophic stage (Fig. 91). In *Iodamoeba* the endosome shows no reaction but the zone of periendosomal granules reacts strongly. In *Endolimax* the endosome of the trophic stage shows no reaction but the peripheral layer gives a

weak reaction. This reaction may be intensified by the use of formalin as a fixing fluid and it is also somewhat intensified in the cysts, at least, by hydrochloric and nitric acids. In *Dientamoeba* the appearance of the nuclei is not essentially different from what it is with the other two stains. In this respect this species is distinctly different from all the others.

In some respects the Feulgen reactions recorded here are different from those recorded by certain other observers. For example, Levitanskaia (1938) reports that *E. coli* has a large reacting karyosome (endosome) in the trophic stage. In the cysts he reports scattered granules as I have found. Both Reichenow (1928) and Levitanskaia state that there is a faint reaction in the peripheral layer of *E. histolytica* but I have not been able to confirm this. Both these authors reported the same results with *Iodamoeba* that I have obtained. Levitanskaia noted the faint reaction of the peripheral layer in the nucleus of *Endolimax* trophic stages but stated that in the cysts only the endosome reacts. I have found the cyst reaction to be variable; some show a reaction in the peripheral area as shown in Fig. 98 while others show a reaction in the central area. It seems probable that when the reacting material appears in the central area, some phase of nuclear division, either prophase or telophase, is involved. My results with *Dientamoeba* agree with those of Levitanskaia.

On the whole, these evidences of chemical differences, especially as between and among the different genera offer important confirmation to the validity of the taxonomic groupings which have been established.

#### SUMMARY

1. The importance of properly prepared slides as an aid to the diagnosis of intestinal protozoa is emphasized.
2. Experiments show that fixation for one minute gives results as good as or better than longer times for the fixing agents commonly employed for intestinal protozoa.
3. Schaudinn's fluid and other fixing solutions can be greatly diluted without destroying their effectiveness as fixing agents. Mercuric chloride, acetic acid, alcohol, etc., in weaker solutions, are better fixing agents than stronger solutions. It is suggested that new fixing agents, much weaker than those commonly employed, may well be devised.
4. Acetic acid does not destroy the stainability of the parabasal body of *Trichomonas muris* for hemalum even when used alone at 20 per cent. Strong concentrations do reduce the stainability of the nuclei of the common intestinal amoebae except *Dientamoeba*. It tends to increase the stainability of the nuclei of that species. Strong concentrations (50 per cent or more) often produce relatively large "buds" on the cysts of *E.*



*histolytica* and *E. coli*. These "buds" seem to lack definite boundaries at their outer margins.

5. As with other organisms, intestinal protozoa sometimes show a surprising amount of variation. Different races of the same species may have different appearances with the same technique. The same race may give different results with the same technique, if the environments are different. Different individuals on the same slide may show different reactions to the same technique.

6. On the other hand, as illustrated by *Trichomonas muris*, different fixing solutions and different stains may produce decidedly different appearances for the same species.

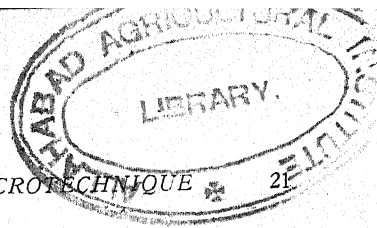
7. Different species show chemical as well as morphological differences in the composition of their nuclei. This is particularly well shown when the Feulgen reaction is applied to the intestinal amoebae of man and the results compared with those obtained with the more common stains.

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#### EXPLANATION OF PLATES

All figures have been drawn from fixed and stained slides. Unless otherwise stated, the stain has been Heidenhain's iron alum hematoxylin. Fixing agents are mentioned in the detailed explanations. Figs. 1 to 10 were drawn originally at  $\times 4000$ . All others at  $\times 3000$ . All have been reduced about one-third in reproduction.

## PLATE I

FIGS. 1 to 5. *Trichomonas muris*, all from the same rat.

FIG. 1. Schaudinn's plus 5% acetic; Heidenhain's. Note deeply stained fibrillar apparatus and cytoplasmic granules.

FIG. 2. Schaudinn's plus 5% acetic; Delafield's. Parabasal stained, granules unstained.

FIG. 3. 20% acetic; Heidenhain's. Fibrillar apparatus swollen but unstained.

FIG. 4. 20% acetic; Delafield's. Parabasal stained.

FIG. 5. 2% chromic acid plus 5% acetic; Heidenhain's. Endosome and costa are only structures stained.

FIGS. 6 to 10. Cysts of *Giardia lamblia*.

FIGS. 6 to 9. Schaudinn's plus 5% acetic. All from one slide. Note variations in staining.

FIG. 10. Schaudinn's plus 5% acetic; phosphotungstic acid hematoxylin. Peripheral layer of nucleus stains deeply, endosomes do not.

FIGS. 11 to 13. Cysts of *Iodamoeba bütschlii*.

FIG. 11. Schaudinn's plus 10% acetic. Endosome stained. With more acetic, endosomes are usually unstained.

FIG. 12. 50% of sat.  $\text{HgCl}_2$ ; hemalum. Endosome faint except for small granule (= centriole?).

FIG. 13. 10% acetic. Very little stain; endosome not sharply differentiated.

FIGS. 14 to 28. *Endolimax nana*.

FIGS. 14 to 17 are from same case.

FIGS. 14 and 15. Schaudinn's plus 5% acetic. Endosomes deeply stained.

FIGS. 16 and 17. Schaudinn's plus 20% acetic. Endosomes faint.

FIGS. 18 to 23 are from another case.

FIGS. 18 to 20. Schaudinn's without acetic. Variable stainability of endosome on same slide.

FIGS. 21 to 23. Schaudinn's plus 20% acetic. Another slide, variable stainability of endosome.

FIGS. 24 and 25. Another case.

FIG. 24. Schaudinn's plus 5% acetic. Appearance typical.

FIG. 25. Bouin's. Shrinkage space around nucleus.

FIGS. 26 to 28. Another case. Fixations are, respectively, Schaudinn's plus 5% acetic; one-half the same; one-fourth the same.

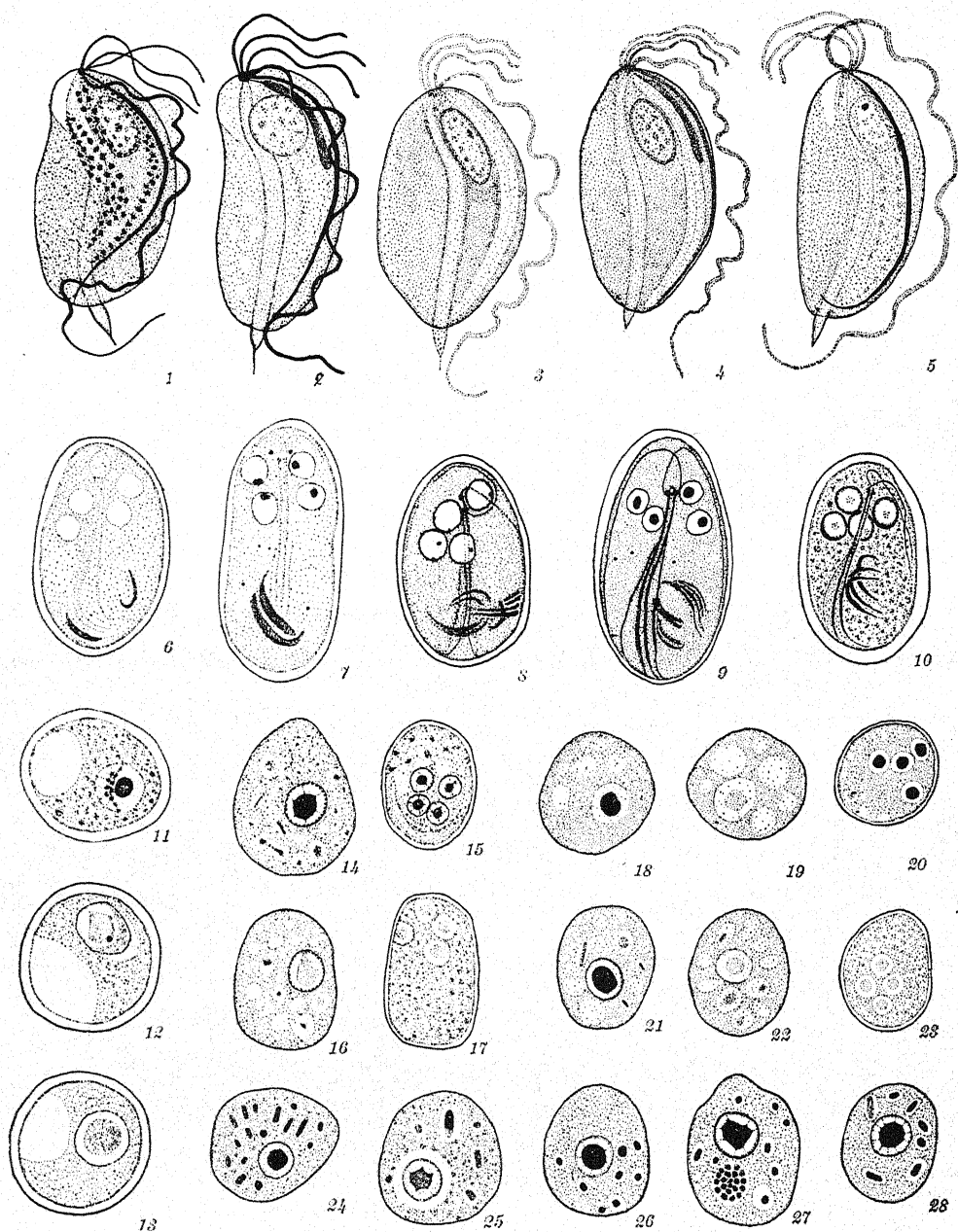


PLATE I

## PLATE II

FIGS. 29 to 40. *Endolimax nana* trophic stages, all from same case; all differentiated with picric except Fig. 39.

FIG. 29. Sat.  $\text{HgCl}_2$ . Endosome not deeply stained.

FIG. 30. Same plus 5% acetic. Endosome deeply stained.

FIG. 31. 50% of sat.  $\text{HgCl}_2$ . Endosome deeply stained.

FIG. 32. Same plus 5% acetic. Endosome lightly stained.

FIG. 33. 50% ethyl alcohol. Endosome darkly stained.

FIG. 34. Same plus 5% acetic. Endosome more lightly stained.

FIG. 35. 70% ethyl alcohol. Endosome darkly stained.

FIG. 36. Same plus 5% acetic. Endosome more lightly stained.

FIG. 37. 95% ethyl alcohol. Endosome darkly stained.

FIG. 38. Same plus 5% acetic. Endosome darkly stained.

FIG. 39. 5% acetic acid. Alum differentiation. Endosome lightly stained.

FIG. 40. Same, picric differentiation. Endosome darker.

FIGS. 41 to 56. *Dientamoeba fragilis*. All picric differentiation except Figs. 51, 53, 54, 55.

FIGS. 41 to 52. Same slides as Figs. 29 to 40. Figs. 29 and 41 from same slide; Figs. 30 and 42 from same slide, etc.

FIG. 41. Sat.  $\text{HgCl}_2$ . Nuclei indistinct.

FIG. 42. Same plus 5% acetic. Nuclear granules stained.

FIG. 43. 50% sat.  $\text{HgCl}_2$ . Nuclei and nuclear granules indistinct.

FIG. 44. Same plus 5% acetic. Nuclear granules stained.

FIG. 45. 50% ethyl alcohol. Nuclear granules indistinct.

FIG. 46. Same plus 5% acetic. Nuclear granules distinct.

FIG. 47. 70% ethyl alcohol. Nuclei fairly well differentiated.

FIG. 48. Same plus 5% acetic. Nuclear granules more distinct.

FIG. 49. 95% ethyl alcohol. Central nuclear area stained but granules indistinct.

FIG. 50. Same plus 5% acetic. Nuclear granules more distinct.

FIGS. 51 and 52. 5% acetic; alum and picric differentiation, respectively; no significant difference.

FIGS. 53 and 54. Another case.

FIG. 53. Schaudinn's plus 5% acetic. Nuclei poorly differentiated.

FIG. 54. Same, one-half strength. Nuclei more clearly differentiated.

FIGS. 55 and 56. Another case; feces acid.

FIG. 55. Schaudinn's with no acetic. Nuclei fairly well stained.

FIG. 56. 100% acetic. Central nuclear area stained, granules indistinct.



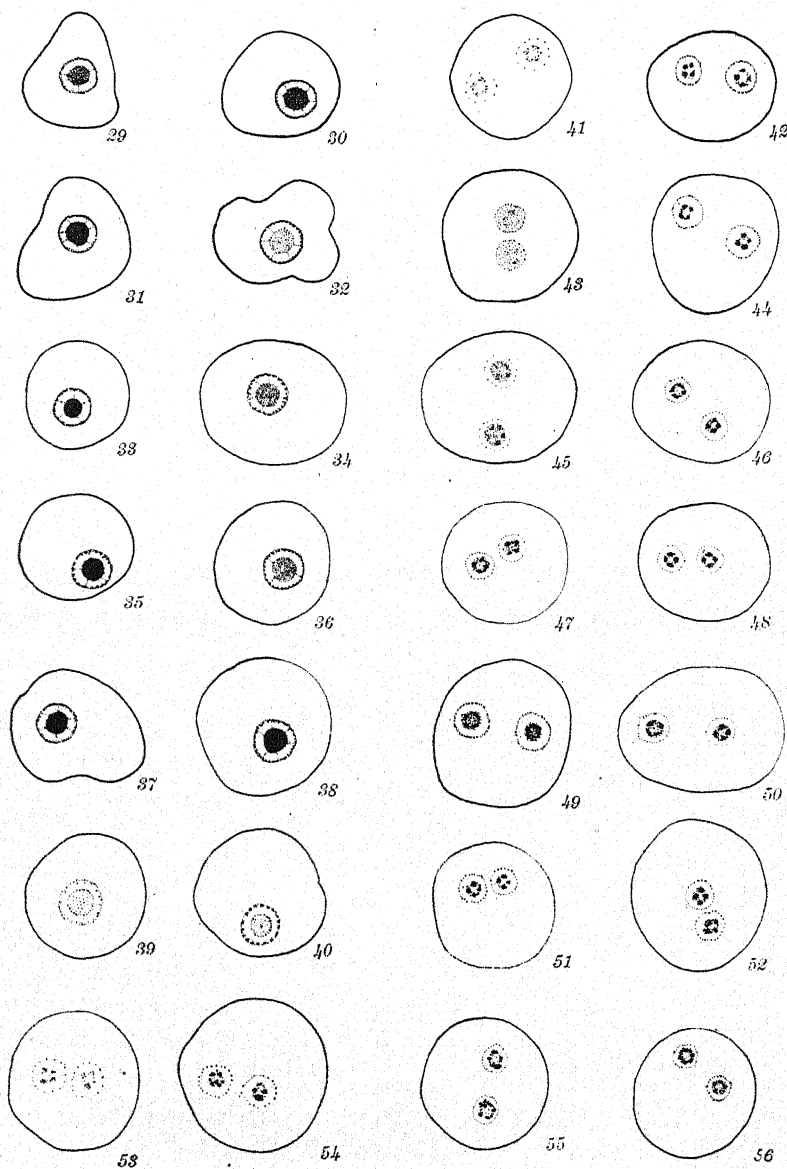


PLATE II

## PLATE III

FIGS. 57 to 72. *Entamoeba histolytica*.

FIGS. 57 to 67. From same case; picric differentiation.

FIGS. 57 and 58. Schaudinn's plus 5% acetic. Trophic stage and mature cyst, typical staining.

FIGS. 59 and 60. 5% acetic. Trophic stage and cyst. Endosome unstained.

FIGS. 61 and 62. 10% acetic. Trophic stage and cyst. A little stain remains in peripheral layer of nucleus.

FIGS. 63 and 64. 20% acetic. Trophic stage and cyst. (Note parasitic *Sphaerita* in cyst.) No stain in nuclei.

FIGS. 65 and 66. 50% acetic. Trophic stage and cyst. A little stain in peripheral layer of trophic stage; broad shrinkage space in cyst.

FIG. 67. 50% acetic. Cyst. Broad extrusion ("bud"), with no definite boundary on outer surface.

FIG. 68. Schaudinn's plus 5% acetic. Another case. A typical "bud" with definite boundary.

FIGS. 69 and 70. Another case, trophic stages.

FIG. 69. Schaudinn's plus 5% acetic. Typical condition of nucleus.

FIG. 70. Allen's B3. Shrinkage area inside nucleus.

FIGS. 71 and 72. Another case, trophic stages (drawings by Mr. R. L. Brown).

FIG. 71. Schaudinn's plus 5% acetic. About usual condition.

FIG. 72. Allen's B3. Endosome unstained. Central nuclear area shows contraction.

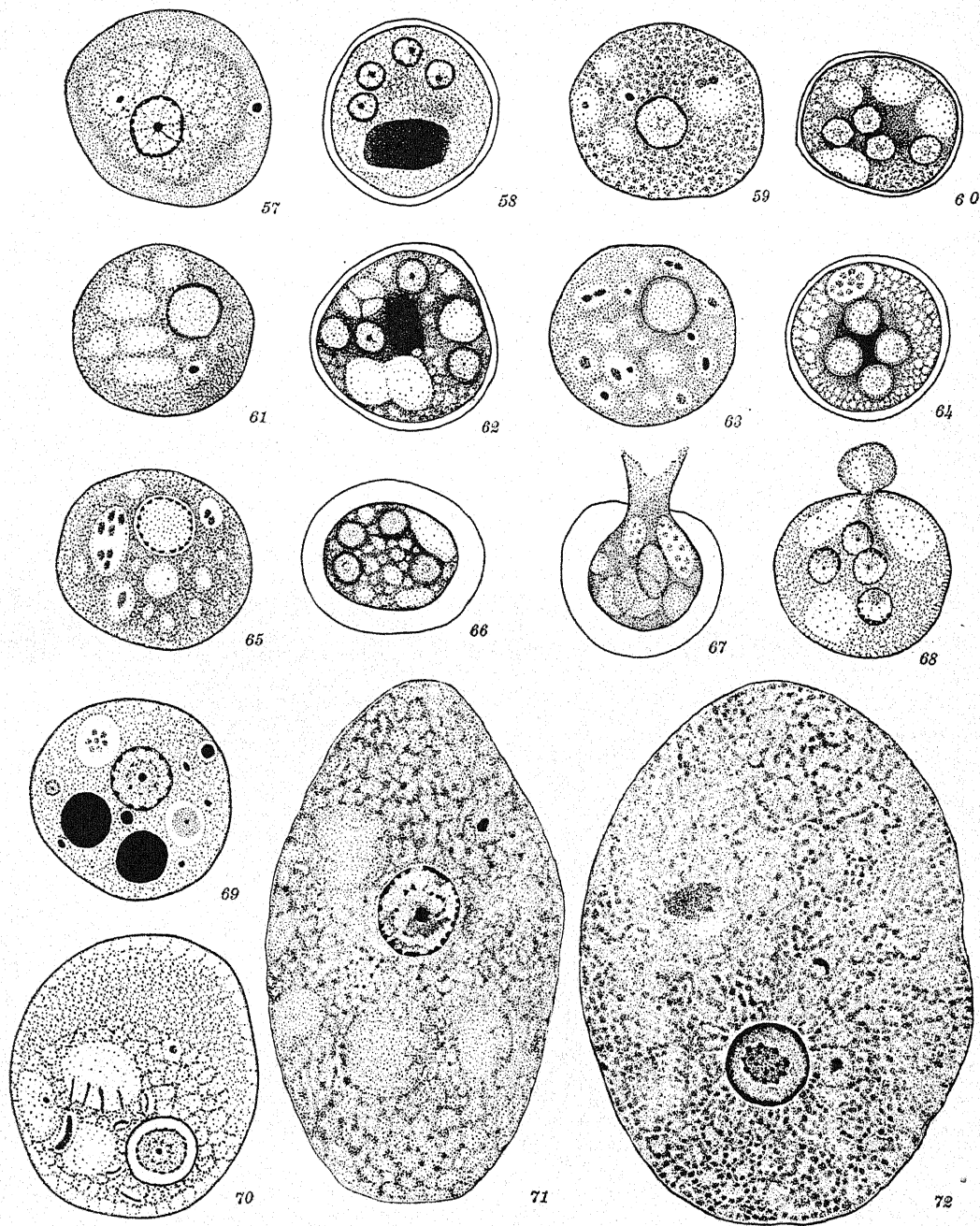
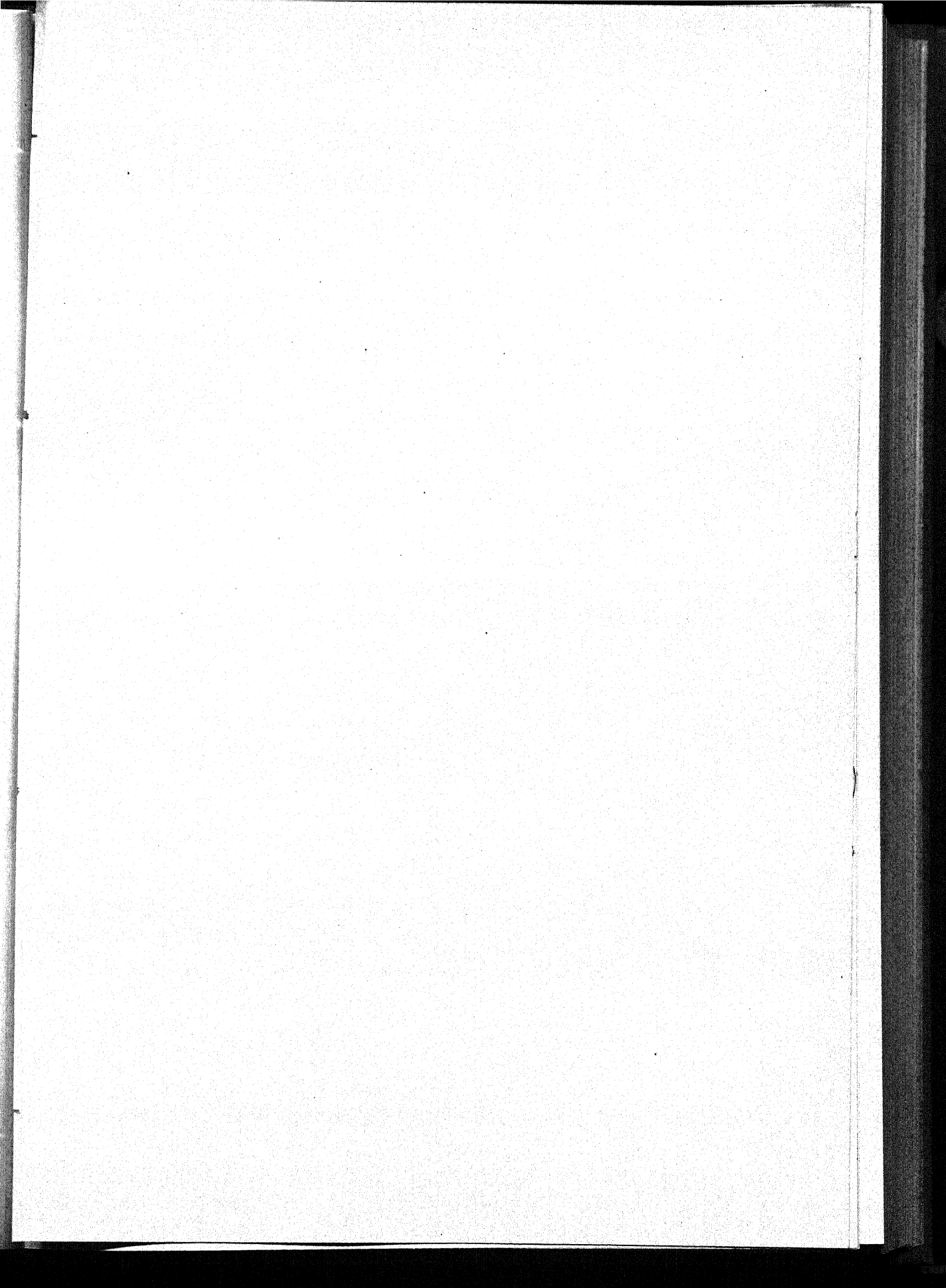


PLATE III

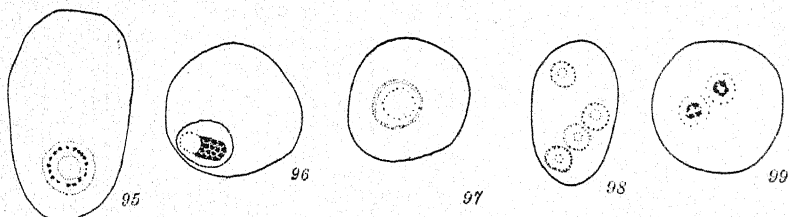
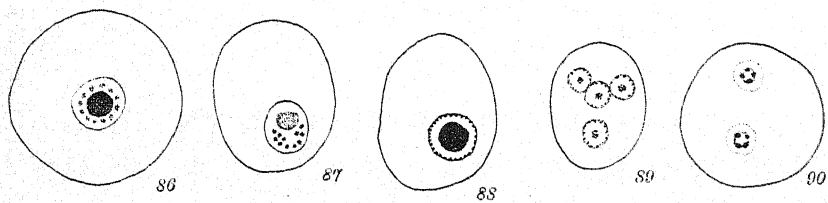
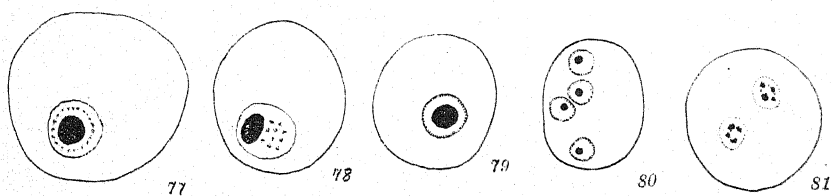
## PLATE IV

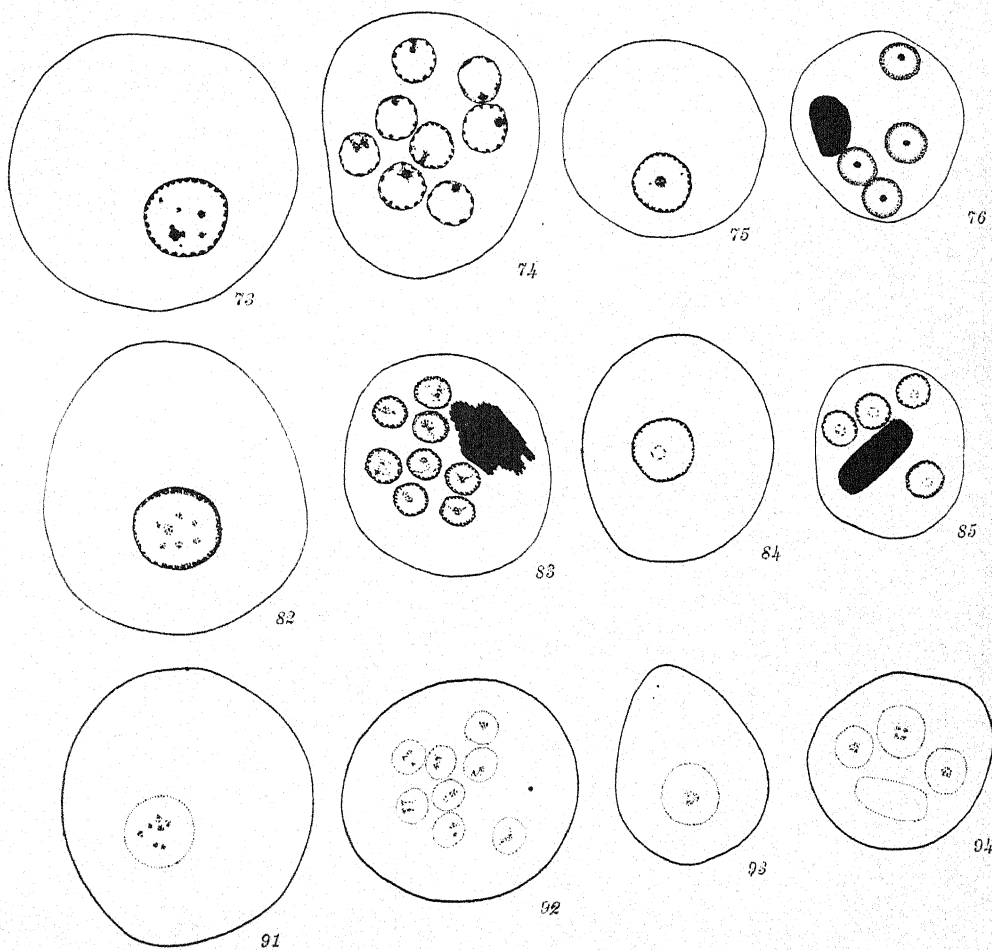
The intestinal amoebae of man. Fixed in Schaudinn's plus 5% acetic. Aside from nuclear and endosomal profiles only deeply stained nuclear materials and chromatoids were drawn. For Figs. 73 to 81, stain is Heidenhain's; Figs. 82 to 90, stain is hemalum; Figs. 91 to 99, stain is Feulgen. Figs. 73, 74, 75, 76, 79, 80 are from one slide; Figs. 82, 83, 84, 85, 88, 89 are from another slide from same case; Figs. 91, 92, 93, 94, 97, 98 are from a third slide from this case. Figs. 77 and 78 are from one slide from another case. Figs. 86 and 87 are from another slide from this case. Figs. 95 and 96 are from different cases. Figs. 81 and 90 are from one case; Fig. 99 from another case.

- Figs. 73, 82 and 91. Trophic stages of *E. coli*.
- Figs. 74, 83 and 92. Cysts of *E. coli*.
- Figs. 75, 84 and 93. Trophic stages of *E. histolytica*.
- Figs. 76, 85 and 94. Cysts of *E. histolytica*.
- Figs. 77, 86 and 95. Trophic stages of *Iodamoeba bütschlii*.
- Figs. 78, 87 and 96. Cysts of *I. bütschlii*.
- Figs. 79, 88 and 97. Trophic stages of *Endolimax nana*.
- Figs. 80, 89 and 98. Cysts of *E. nana*.
- Figs. 81, 90 and 99. *Dientamoeba fragilis*.











# THE OCCURRENCE OF HELMINTHS AND COCCIDIA IN PARTRIDGES AND PHEASANTS IN DENMARK

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An investigation of the food and parasites of game birds was initiated in October 1936 under the direction of Prof. M. Christiansen of the State Veterinary Serum Laboratory and Prof. R. Spärck of the University of Copenhagen. This investigation was under the auspices of the Jagtfond (Game Foundation) of the Ministry of Agriculture.\*

Since that time 143 adult and 56 partridge chicks (*Perdix perdix*) and 169 adult and 67 pheasant chicks (*Phasianus colchicus*) have been sent in for examination. They came from diverse localities at different times of the year. Not all were examined. The methods of collecting were improved during the investigation. The data concerning the numbers of each species are quantitative. It is felt that they present an accurate picture of both of the various parasites involved and the distribution thereof. Although the number of birds examined is small, several interesting points have been revealed. The numbers of certain parasites in pheasants are higher than in partridges and also the variation in the number of certain parasites with age differences of the hosts.

## MATERIALS AND METHODS

The helminths were collected under low magnification with a microscope; the trachea, crop, and proventriculus were examined directly. For the crop this proved to be an unsatisfactory method. In consequence *Capillaria contorta* was not observed during the investigation when the crop was submitted to a formalin solution. The contents of the small intestine were examined through 1 and 0.5 mm wire mesh screens and the washings passed through a 0.25 mm sieve. The supernatant fluid was poured off and the sediment suspended in 50 per cent sugar solution and examined. Samples of the surface film were then taken when necessary. The present female spec-

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\* I wish to express my thanks to the Ministry of Agriculture for their help in this investigation; also to Dr. G. L. Graham for his criticism of the manuscript.

for calculation of total numbers. With caution these data may be utilized in estimating the density of infections.

#### RESULTS

Although an acanthocephalan, cestodes, trematodes, and nematodes were encountered during the course of this study, only the last were commonly occurring parasites. The others were of infrequent occurrence and infections were invariably light.

##### *Acanthocephala*

A single specimen of *Prosthynchus transversus* (Rud., 1819) was recovered from an adult partridge. The parasite was also observed once in an adult pheasant. This acanthocephalan is a common parasite of various passerine birds but has been previously recorded as a rather rare parasite of pheasants and partridges in England by Clapham (1938).

##### *Cestodes*

nine pheasant chicks; no adults were so parasitized as adults were infected, although only in most part the worms were in a bad state of preservation to identify. When they could be determined they were *Railletina echinobothridia* (Megnin, 1880). This form in English pheasants and partridges.

##### *Trematodes*

recovered from the intestine of two partridge pheasant chicks. None were found in older birds. The first report of echinostome parasites from pheasants (6) has reported *Echinostoma revolutum* from pheasants. That the worms herein reported are this species.

##### *Nematoda*

the most common helminths encountered in pheasants. The variety of species involved and the degree of infection while some species were either of such rare occurrence or in such small numbers that they could not be determined, others were serious parasites, particularly in pheasant chicks as in young adults.

(Stegeman, 1811). This nematode was comparatively common in pheasants. In the latter it was found only once in an adult bird. In partridges it was found in chicks, although it was an occasional parasite in adults as a rule. This parasite is commonly reported to have been incriminated by Christiansen (1935) as an important helminthic disease in pheasantries.



Apparently *S. trachea* is a more frequent as well as a more serious parasite of game birds in England than in Denmark, if one may judge from various reports (Clapham, 1935, and others).

*Dispharynx spiralis* (Molin, 1858). This parasite of the proventriculus showed, like *Syngamus trachea*, differences in the frequencies with which it was found in pheasants and partridges. In the former it appeared only in chicks and the infections were light. In partridges on the contrary, it was of more common occurrence in both chicks and adults but was encountered most often and in the largest numbers in young adult birds. It is notable that the heaviest infections were concentrated in birds from one definite area, thus suggesting the existence of locally favorable factors for the parasite. This species has not previously been noted from partridges in Europe. It is curious that this relatively common parasite of game birds in Denmark has apparently not been encountered in the extensive investigations on the parasites of game birds in England. Cram (1931) has reported it from *P. perdix* in the United States and also from pheasants (1928).

*Capillaria contorta* (Creplin, 1839). According to Cram (1936) this parasite is known from a large number of birds including partridges and pheasants in which it occurs in the ingluvies and esophagus. As already noted, the direct examination of this portion of the digestive tract did not reveal the presence of this nematode during the first part of this investigation. The parasites were first observed when the infected tissues were submerged in physiological saline. They were only found in pheasants but were probably overlooked in partridges. Apparently they are common, for eight of 11 birds examined were infected. This parasite has not been reported from partridges or pheasants in England.

*Capillaria longicollis* (Rud., 1819). This nematode is a not infrequent parasite of both pheasants and partridges, occurring in about 25 per cent of those examined. The infections seemed to be slightly heavier in pheasants than in partridges and heaviest during the summer months.

*Capillaria columbae* (Rud., 1819). *C. columbae* is here reported from partridges for the first time. It was encountered on four occasions, in both chicks and older birds from widely separated areas at all times of the year in light infections. It was never found in pheasants.

*Capillaria collaris* (von Linstow, 1873). It is only with hesitation that these cecal parasites are referred to the species described by von Linstow. In most respects they agree very well, especially concerning the spicule and spicule sheath of the male. In the present female specimens there is a protruding membranous appendage of a form differing from that of *C. longicollis*. According to Morgan (1932) and Freitas and Almeida (1935) this is characteristic only of immature females of *C. collaris*. The same observation was made by Koffman (1939) who

distinguished *C. retusa* Railliet and *C. collaris*, although these species are considered synonymous by Freitas and Almeida (1935). Curiously Koffman reported that *C. collaris* was recovered from the small intestine instead of its normal habitat, the cecum.

There was a marked difference in the frequency with which this parasite occurs in adult partridges and pheasants, three per cent in the former, 75 per cent in the latter. In the pheasants, the density of infection was appreciable, whereas in partridges the infections were very light. Oddly, both the incidence and degree of infection with *C. collaris* was very low in pheasant chicks. This is the first report of *C. collaris* from pheasants.

*Ascaridia compar* (Schränk, 1790). While this nematode is commonly reported from partridges and a number of other gallinaceous game birds, it has never been reported from pheasants. In the present investigation it was encountered only twice from partridges. It is obviously not an important partridge parasite, although it is a frequently occurring nematode in game birds other than partridges and pheasants in Norway and Sweden. Possibly *A. compar* is, as suggested by Baylis (1936), a synonym of *Ascaridia galli*.

*Heterakis gallinae* (Gmelin, 1790). This nematode was the most commonly occurring helminth in chicks and adults of both hosts from all localities studied. The infections in chicks tended to be slightly heavier than in adults; the incidence of the parasite in pheasants was twice that observed in partridges, 81 and 40 per cent respectively. The infections in the former were ten times as heavy as those in the latter host.

*Trichostrongylus tenuis* (Mehlis, 1846). This important parasite was present in about 20 per cent of the adult partridges and pheasants examined although both the incidence and degree of infection seemed to vary with different localities. The heaviest infections were observed during the summer months, although no massive infections such as are reported for fatal cases of trichostrongylosis were ever encountered. It seems reasonable nonetheless to consider this parasite potentially dangerous for Danish pheasants and partridges. The wide general distribution of the parasite might lead, under favorable environmental circumstances, to a serious epizootic.

#### *Coccidia*

Altogether there were seven species of coccidia encountered, four in partridges and three in pheasants, although for present purposes they will be treated en masse. They were naturally of very common occurrence and were markedly more frequent in chicks and young birds than in older birds. As with most of the other parasites which have been enumerated, coccidia were more common in pheasants than in partridges and the infections were likewise heavier in the pheasant. Christiansen

(1935-36) has already indicated that coccidiosis is a well known cause of disease in both wild and pen-reared pheasant chicks in Denmark.

In Table 1, the frequency of infection of the two hosts with the various species of parasites is shown as a percentage for adult birds and chicks.

TABLE 1.—The frequency with which Danish partridges (143 adults and 56 chicks) and pheasants (169 adults, 67 chicks) were parasitized with various species of helminths and coccidia. The infections encountered are expressed as a percentage of the number of birds examined

	Partridges Per cent parasitized		Pheasants Per cent parasitized	
	Adults	Chicks	Adults	Chicks
<i>Acanthocephala</i> .....	1	0	1	0
<i>Cestodes</i> .....	2	7	0	13
<i>Echinostomes</i> .....	0	4	0	3
<i>Nematoda</i> .....				
<i>Syngamus trachea</i> .....	5	0	1	10
<i>Dispharynx spiralis</i> .....	9	6	0	3
<i>Ascaridia compar</i> .....	2	0	0	0
<i>Capillaria longicollis</i> .....	20	26	23	28
<i>C. contorta</i> .....	4	2	73*	0
<i>C. columbae</i> .....	4	2	0	0
<i>C. collaris</i> .....	3	2	75	6
<i>H. gallinae</i> .....	40	52	81	74
<i>Trichostrongylus tenuis</i> .....	24	2	22	6
<i>Coccidia</i> .....	48	62	82	73
Not parasitized .....	20	13	2	24

\* Only 11 birds examined.

#### DISCUSSION

Although much of the information which has been gained from this study of the parasitic fauna of Danish pheasants and partridges cannot be considered as new, a knowledge of the variety of parasites involved as well as information on their relative frequency and parasitic density was a necessary prerequisite for further work. Granting that parasites may condition to some extent game management and control problems, the present investigation, even though based on the examination of a comparatively small number of birds, provides a basic quantitative background not previously available for Danish game birds.

The indications that the relative importance of certain parasites may here differ considerably from those prevailing in other game areas, as for example in England, seem particularly important. Interesting also is the fact that while the parasites tabulated in Table 1 are for the most part common to both pheasants and partridges, the frequency of their occurrence in some instances indicated a variation in the apparent favorableness of these hosts for these specific parasites. Thus *Dispharynx spiralis* occurred more commonly in partridges than in pheasants, whereas *Capillaria collaris*, *Heterakis gallinae*, and coccidia occurred more often in pheasants. Others, such as *Ascaridia compar* and *Capillaria columbae*, occurred exclusively in one host; still others, such as *C. longicollis* and *T. tenuis* occurred with about equal frequency in both hosts.

The most pronounced difference attributable to age of the host occurred in the case of *Trichostrongylus tenuis* in both pheasants and partridges, the chicks being both infrequently and lightly infected in comparison with the adult birds. On the other hand, in the case of the echinostomes and cestodes, the infections were almost always in chicks and rarely in adults. Age differences with other parasites were suspected, but the limited data do not justify definitive statements.

In conclusion it may be observed that the data offer a glimpse of a complex biological and ecological relationship which must certainly vary from season to season and contain elements which need to be more fully understood in order to ensure maximum numbers of these game birds over their range. In some places game keeping is practiced intensively.

#### SUMMARY

A total of 143 adult and 56 partridge chicks (*Perdix perdix*) and 169 adult and 67 pheasant chicks (*Phasianus colchicus*) from various game keeping areas in Denmark have been examined for internal parasites. In addition to coccidia, there were found an echinostome, cestodes, an acanthocephalan and nine species of nematodes. The echinostome, apparently *Echinostoma revolutum*, has not heretofore been reported from pheasants. The appearance of *Dispharynx spiralis* in partridges seems to be a new European host record. *Capillaria columbae* from partridges and *C. collaris* from pheasants are new host records for these parasites.

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STUDIES ON THE LIFE HISTORY OF *EUPARYPHIUM*  
*MELIS* (TREMATODA: ECHINOSTOMIDAE)<sup>1</sup>

PAUL C. BEAVER

On August 8, 1938, an undescribed species of echinostome cercaria was found in one of 133 adult *Stagnicola emarginata angulata* collected from North Fishtail Bay on Douglas Lake, Michigan. Its behavior and morphology were recorded and certain facts concerning the metacercaria were obtained. On August 10 of the following summer, another snail infected with this species was discovered. On this occasion 550 *S. emarginata angulata* were taken from near the first location at Nutting Bay on Douglas Lake. From experiments on the material thus obtained, the redia, cercaria, metacercaria, and adult stages were determined for a species which seems to be identical with *Euparyphium melis* (Schrunk, 1788).

THE CERCARIA

(Figs. 7, 8, 9)

The cercariae emerge between 5 and 9 PM, and live about 24 hours in the laboratory. They swim and creep with comparatively great speed, and characteristically are nearly equally distributed throughout the container until they become spent and settle to the bottom. During the period soon after emergence, they alternately creep and swim in intervals of 2 to 3 minutes. In behavior and general appearance, this cercaria is strikingly similar to the cercaria of *Psilostomum ondatrae* Price (Beaver, 1939b). Contracted, the body is somewhat angular in outline with the width and length about equal; extreme extension brings its width to less than one-eighth its length. The tail may contract to one-third its greatest length. The cercariae readily detach the tail when put under pressure of the coverglass and are crushed more easily than most echinostomes.

*Description:* Body relatively transparent, colorless; elongate but with stout proportions, the post-acetabular region slightly set-off from the remainder; greatest width in relaxed condition near middle of pre-acetabular region. Cuticula thick but relatively soft, bearing extremely minute spines anteriorly, and extending beyond margins of suckers to form a transparent ruffle on the border of each. Collar spines easily seen, distributed as follows: 4 angle spines, 6 laterals in a single row, and 7 dorsals in a double row, 4 of the dorsals being oral. Angle spines much larger than

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the others. Although the spines are well developed the collar itself is almost indistinguishable.

Oral sucker terminal, or slightly subterminal, muscular. Width of pharynx one-third that of oral sucker. Acetabulum in posterior third of body, very muscular, slightly protrusible, somewhat larger than oral sucker. Gut without lumen but evident in both living and fixed preparations. Excretory bladder with large posterior chamber and smaller anterior one, both contractile. Excretory siphons arise mesially from bladder, extend much coiled to sides of acetabulum where they describe a sharp lateral loop, then become inflated as they form three (sometimes 4) rounded loops containing 15 to 25 large scattered granules along sides of esophagus. Before reaching level of pharynx siphons become small and straight; at level of angle spines they reverse their course and extend unbranched to extreme posterior end of body where branching occurs. Vibratile tufts in the latter are about 10 in number. Flame cells 17 or 18 on each side, apparently arranged in 6 groups of 3 each, half above and half below the acetabulum. The 3 in the region of the oral sucker are definitely arranged in a single group, the others appear to be so arranged.

Portion of body above level of pharynx very clear; remainder of body more granular due to angular masses of finely granular cystogenous material. Six pairs of large nuclei appear along sides of esophagus in stained specimens. No cephalic ducts were observed. Tail terminal, plain, having no fins or folds, and provided with both longitudinal and circular muscles to the extreme tip. Caudal excretory tube extends through first fourth of tail, bifurcates, and attaches to, but does not empty through, the lateral wall.

Measurements on fixed material extended as in Fig. 7 are: body length, 0.384 mm; tail length, 0.545 mm; body width at crown, esophagus, and acetabulum respectively, 0.08 mm, 0.18 mm, 0.15 mm; greatest width of tail, 0.052 mm; oral sucker, 0.060 by 0.054 mm; pharynx, 0.024 by 0.018 mm; esophagus, 0.169 by 0.012 mm; acetabulum, 0.054 by 0.078 mm; angle spines, 18–20  $\mu$  by 2–3  $\mu$ ; dorsal spines, 12–14  $\mu$  by 2–3  $\mu$ . The following additional measurements were made on living material under a coverglass: diameter of siphon granules, 4–14  $\mu$ ; greatest extension of body about 0.5 mm; tail, 0.55 by 0.06 mm; acetabulum, 0.09 by 0.08 mm; oral sucker, 0.08 by 0.07 mm; length of flame cells, 2  $\mu$ .

#### THE REDIAE

(Figs. 10, 11)

The infections were old and both of the snails examined had been in the laboratory more than three weeks. They were alive when opened, however, and although only a few cercariae were emerging during the period immediately preceding the examination, an abundance of active rediae of various sizes were obtained from the liver and gonad. The long period in the laboratory may account for the presence of not more than 3 mature cercariae in the largest rediae.

*Description.* Living specimens very active, especially younger ones; body long, slender, sharply tapered at posterior end; colorless even in oldest individuals. Appendages unusually long and motile, attachment distinctly ventral; distance from posterior end to appendages about one-fourth total body length but varies between one-fifth and three-fifths. Collar well developed, motile, conspicuous in all states of contraction and after fixation; completely divided into 4 lobes, a dorsal, a ventral, and 2 laterals; located well back of anterior end, half-way between oral sucker and birth-pore. Birth-pore dorsal and usually very conspicuous. Sucker ordinary. Gut large, often quite filling the body, and extending well beyond appendages. Measurements on well extended fixed material: average mature specimens, 1.4–1.7 mm in length; sucker, 56 by 50  $\mu$  to 80 by 60  $\mu$ . Excretory bladders somewhat nearer appendages than collar; two groups of flame cells on either side, each containing 24 or

more flame cells in specimens around 1.5 mm in length; anterior pair located just posterior to collar, the other one at base of appendages. No mother rediae were observed although immature rediae that apparently had recently emerged were numerous. The smallest of these was 0.32 mm in length.

#### THE METACERCARIA

(Figs. 12, 13)

Under pressure of the coverglass cercariae often lose the tail and form imperfect cysts. Normal encystment could not be induced, however. When isolated with various species of fish (*Ameiurus nebulosus*, *Perca flavescens*, *Umbra limi*, *Lepomis pallidus*, *Ambloplites rupestris*, and *Percina caprodes*) some of the cercariae would spend short periods creeping on them and a small percentage would encyst in the nostrils and cloaca. This was observed more often in *Ameiurus nebulosus* than any other species of fish.

When isolated with tadpoles, the cercariae behave in a very different manner. They are quick to settle on the tadpoles, begin rapid creeping immediately, and enter the cloaca in a remarkably short period. Their behavior in this respect is identical with that previously described for *Echinostoma revolutum* (Beaver, 1937) in which the direction taken is ventro-posterior towards either the cleft between the body and tail or the ventral margin of the tail, and from there directly to the cloaca. Now instead of following the mesonephric ducts forward as is the case in *Echinostoma revolutum*, *Echinoparyphium recurvatum*, *Nephrostomum ramosum*, and some other species of echinostomes, they penetrate the cloacal wall, migrate a short distance through the tissues, and encyst either in the skin or subcutaneous tissues. Occasionally a cyst is found in the deeper connective tissues. The cyst is almost spherical and extremely thin-walled when formed. The wall remains thin in cysts located in the epidermis, but in the dermis and deeper tissues a heavy fibrous wall of host tissue is added in 5 or 6 days. Heavily infected tadpoles are readily recognized by local swelling and rough thick skin in the cloacal region (Fig. 13). Exclusive of host tissue, the cysts vary from 0.152 by 0.145 mm to 0.170 by 0.170 mm. The true cyst-wall is 2–3  $\mu$  thick and the host tissue after 37 days was found to be 0.120–0.133 mm thick on cysts that were removed from the host.

During the first three weeks in the cyst there is continuous growth in the metacercaria, and noticeable changes in its morphology occur. As it increases in size the body becomes flexed ventrally, and the suckers and pharynx, especially the latter, become proportionately larger than in the cercaria; the gut develops a lumen; the siphon granules remain the same in number and size but extend into the post-acetabular region; the cuticular spines become relatively large and conspicuous; and the collar itself becomes more pronounced. The following measurements were made

(without pressure) on a living specimen 21 days old: diameter of acetabulum,  $90\ \mu$ ; oral sucker,  $70$  by  $60\ \mu$ ; pharynx,  $36$  by  $30\ \mu$ ; length of dorsal collar spines,  $18$ – $20\ \mu$ ; length of angle spines,  $24$ – $28\ \mu$ ; body extended,  $0.42$  by  $0.16$  mm.

The metacercaria is probably infective after three weeks. At any rate, there is no further noticeable development in stages up to 37 days of age.

#### THE ADULT

(Figs. 1–6)

Metacercariae under 6 days of age were fed to a rat, 2 mice, a cat, and a pigeon. All were negative when examined 3 and 8 days after feeding. It was later observed that the metacercariae were undergoing development in the cysts and because experimental material was relatively scarce, no further attempts were made to obtain adult worms until the metacercariae were 3 weeks old. Then 2 kittens and a ferret were fed infected tadpoles. The kittens were each given 2 tadpoles carrying about 50 cysts. Both were negative when examined after 5 and 8 days. The ferret was given 5 tadpoles carrying about 100 metacercariae, 26 to 37 days of age. It was fed nothing but fresh pork liver and canned fish. A severe diarrhea developed on the 9th day and persisted through the 10th and 11th days. Because unhealthy animals frequently lose intestinal worms, and especially because it was observed that the ferret had eaten about 30 square inches of a cotton towel, it was thought advisable to terminate the experiment. One immature worm was found high in the duodenum. About 2 hours later, after this worm had been studied, described, and fixed, the stomach was examined. Most of the toweling was still in the stomach. No worms were found.

The tadpoles used in these experiments were collected from an acid lake where snails do not occur, and where many other tadpoles have consistently been found by numerous investigators to be negative for larval trematodes. There seems to be no doubt about the history of the worm found in the ferret. The cephalic spination is identical in the cercaria, metacercaria, and adult. The character of the suckers, cuticula, and body proportions are likewise similar in the metacercaria and adult.

Because the experimental worm is immature, some of the taxonomically important features of the echinostomes, such as vitellaria, ova, and cirrus sac complex, can not be described for it (Fig. 6). Other structures, however, are well formed and enough of its specific characters are evident to make its identification positive.

*Description:* Body fusiform, rather stout in moderate state of contraction. Length,  $1.46$  mm; width at acetabulum,  $0.348$  mm; width of collar,  $0.18$  mm. Oral sucker sub-terminal,  $0.09$  by  $0.84$  mm; pre-pharynx short; pharynx,  $0.070$  by  $0.056$  mm; esophagus and ceca simple, the latter reaching almost to posterior end. Acetabulum powerful, situated above middle of body,  $0.204$  by  $0.210$  mm. Testes median,

half way from acetabulum to posterior end, round. Ovary immediately anterior to testes, slightly dextral, round. Uterus short, coiled transversely. Cirrus sac pyriform, widest anteriorly, dorsal to and in front of the anterior part of acetabulum. Cuticula thickly set with minute spines on anterior half of ventral side and on pre-acetabular portion of dorsal side. Collar not pronounced but clearly evident; spines arranged as in cercaria and metacercaria; angle spines about 37 by 6  $\mu$ , dorsal spines about 32 by 5  $\mu$ . Excretory pore distinctly dorsal.

There are two known species that closely resemble the experimental worm. *Euparyphium melis* (Schränk, 1788) is found in several species of European MUSTELIDAE (*Mustela foina*, *Lutra vulgaris*, *Meles taxus*) (Dietz, 1910) and also has been reported from the mink (*Mustela vison*) in North America (Law and Kennedy, 1932). *Euparyphium inerme* (Fuhrmann, 1904) occurs in the otter (*Lutra* sp.) in Java, and has been reported once from North American mink (Price, 1932). Other species which have been placed in the genus *Euparyphium* are: *E. jassyense* Leon and Ciurea, 1922, *E. capitaneum* Dietz, 1909, *E. incrassatum* (Dies.), *E. suinum* Ciurea, *E. spiculator* Obitz, 1933, *E. guerreroi* Tubangui, 1931, *E. ilocanum* (Garrison, 1908), *E. murinum* Tubangui, 1931, and *E. malayanum* (Leiper, 1911).

Through the kindness of other collectors, several good series of worms were received from the mink and otter in this country and from the otter in Java. The material from Java corresponds in nearly all details with the description of *Euparyphium inerme*. Cuticular spines were absent in Fuhrmann's specimens and spines of the cirrus were not mentioned in the description. Both are present in my specimens and they all have 27 instead of 26 collar spines. In spite of these differences in observations, it seems probable that the two collections are of the same species. Neither the experimental worm nor any of the series from North American hosts can be *E. inerme*. In the latter, the testes are extremely long and winding in older worms, and are very elongate in immature ones. The collar spines are longer and more slender, the acetabulum is relatively larger, and the body is more compressed dorso-ventrally in *E. inerme*.

The material from this country is without question the same species as that reported from the mink by Law and Kennedy (1932) and described as *Euparyphium melis* (Schränk). Comparison of the European form (based on Dietz, 1910) with the North American specimens reveals a few possible points of difference. In the former, the 27 collar spines are rounded at both ends and 10 of them are described as oral. The accompanying text figure shows but 4 distinctly oral, however. In well fixed specimens of my collection, all spines of the collar are pointed at the outer ends and 4 of the dorsals are oral in position. That these differences are not real, but rather are due to different post-mortem histories and different interpretations of the structures observed, can not be demonstrated now. The two collections are so similar in other respects that a new name



for the American species probably would be rejected as soon as the European form is better known.

Since it is not certain that the European and American forms are identical, and no taxonomic description has been published for the latter, such a description is here given, based on whole mounts and sectioned specimens from the mink collected in Michigan and Minnesota, and from the otter collected in Michigan.

*Euparyphium melis* (Schränk)

*Description:* Body elongate, rounded at both ends; sides tapering anterior to acetabulum, more parallel in hind body. Mature worms range between 3.86 by 0.65 mm and 10.5 by 2.1 mm, with the greatest width at or just back of the acetabulum. Cuticular spines very minute, extending to acetabulum dorsally and to testes ventrally. Oral sucker sub-terminal, walls thick, shape pyriform with narrowest part posterior; its posterior border reaching level of angle spines. Crown moderately developed, well set off in living worms and well fixed ones, but much less so in worms fixed post-mortem. Collar spines large, rather stout, abruptly tapered at outer end, widest level often just proximal to taper; 27 in number with 4 on each angle, 6 in a single row on each side, 4 orals and 3 aborals on the dorsal side. Angle spines in 2 pairs, one over the other, the deeper or more posterior pair being somewhat larger than the other. Lateral spines about equal, and generally much smaller than angle spines. Dorsals slightly larger than laterals but smaller than those of the angles; orals slightly larger than aborals. Pharynx ovoid, length about two-thirds diameter of oral sucker. Neck narrower than crown in extended worms, broader in contracted ones. Bifurcation of esophagus immediately in front of acetabulum. Wall of esophagus same as in ceca. Acetabulum almost spherical, with thick walls; depth usually less than diameter; located far above middle of body.

Testes tandem, usually contiguous; tend to be plain and round, become elongate in older worms and may have 1 to 4 shallow notches in the margins; their location varies with length of worm but tends to be nearer acetabulum than posterior end. Cirrus sac elongate, extending just beyond middle of acetabulum in some specimens. Seminal vesicle long, slender, straight; divided into large posterior and smaller anterior chambers; wall of anterior portion thick, containing 30 to 36 muscle bands which are very prominent in transverse sections. Pars prostatica about same length as muscular portion of seminal vesicle; prostate gland moderately developed. Cirrus very long, much coiled when withdrawn; length about 0.8 mm in specimens 5-6 mm long; spines on cirrus thickly set, about  $2\mu$  long; evident in sections or when extended, and may be seen inside of cirrus sac in some specimens.

Ovary regular in outline, spherical to pyriform, located on right side and slightly ventral. Oviduct arises from dorsal side. Laurer's canal well developed with large lumen and median dorsal opening to outside. Mehlis' gland broadly oval with sharp regular margins; compact cells of two distinct types, larger basophil proximal cells and more numerous but smaller neutrophil cells. The most proximal portion of uterus forms seminis receptaculum uterinum; true seminal receptacle absent (Fig. 5). Uterus short, forming 3 to 5 transverse coils back of acetabulum. Metraterm on left side of cirrus, not especially muscular and not well set off from uterus; opening of metraterm back of cirrus. Vitellaria extend from level of anterior margin of ovary to posterior end of body, the two fields confluent along dorsal side and to a less extent along ventral side back of testes; often there are almost no follicles along mid-ventral side. Vitelline follicles irregular in shape, 40-120  $\mu$  in diameter. Vitelline ducts pass ventral to ceca and enter vitelline reservoir separately; reservoir well developed, situated dorsal and posterior to remainder of female complex. Ova large, with heavy shells, amber in color; 117 by 72  $\mu$  to 130 by 84  $\mu$ . Excretory pore sub-terminal, distinctly dorsal.



Measurements on average specimen: length 6.1 mm; width at acetabulum, 0.82 mm; width of collar, 0.52 mm; oral sucker, 0.282 by 0.291 mm; pharynx, 0.198 by 0.148 mm; distance between suckers, 0.68 mm; diameter of acetabulum, 0.214 mm; cirrus sac, 0.602 by 0.260 mm; distance between acetabulum and ovary, 0.300 mm; ovary, 0.256 by 0.215 mm; Mehlis' gland, 0.300 by 0.215 mm; anterior testis, 0.563 by 0.382 mm; posterior testis, 0.700 by 0.420 mm; length of post-testicular region, 2.75 mm; ventral angle spines, 100 by 32  $\mu$ ; dorsal angle spines, 120 by 38  $\mu$ ; third lateral spine, 80 by 26  $\mu$ ; second oral dorsal spine, 104 by 32  $\mu$ ; middle aboral spine, 98 by 28  $\mu$ ; body spines at level of genital pore, 18  $\mu$ .

Hosts: *Mustela vison* and *Lutra canadensis*.

Location: Stomach and upper duodenum.

Distribution in North America: Michigan, Minnesota, Ontario.

Museum specimens: U. S. Nat. Mus. Helm. Coll. Nos. 36686-36691.

Autopsy records give both the stomach and duodenum as sites of infection. The experimental worm reported here was taken from the duodenum near the pylorus. None was found in the stomach although several may have been present and overlooked because of the unfavorable conditions under which the examination was made.

#### DISCUSSION

The life cycle of *Euparyphium melis* differs markedly from all life cycles yet determined for apparently closely related species. *Euparyphium ilocanum* as determined by Tubangui and Pasco (1933) differs by having a redia with a complete collar and short gut, a cercaria with narrow well-filled siphons and pronounced collar, a metacercaria that occurs in snails in heavy-walled cysts, and an adult with very numerous collar spines, long uterus, and vitellaria reaching far above the ovary. *Euparyphium murinum* as determined by Tubangui (1932) differs in exactly the same characters as *Euparyphium ilocanum*, having only 45 to 46 collar spines instead of 51, however. Rao (1933) did feeding experiments with metacercariae in snails and fish from which he obtained adult echinostomes in two dogs and a kitten. He regarded these adult worms as identical with *Euparyphium malayanum* (Leiper, 1911). Since both snails and fish were fed to the experimental animals, the origin of the worms is uncertain.

The four-lobed or broken collar of the redia in *E. melis* is the third of its kind to be described within the past year. The other two are *Psilostomum ondatrae* and *Petasiger nitidus* (Beaver, 1939a, 1939b). Since many other echinostome and psilostome rediae have been described as having a complete collar, it was thought good to reexamine the collar of all forms available. It was found that the rediae of *Echinostoma revolutum*, *Echinoparyphium recurvatum*, *Stephanoprora* sp., *Echinochasmus* sp., a 35-spined echinostome (apparently *Cercaria limbiifera* Seifert), and *Hypodaerium* sp. have the simple complete collar.

The purpose of the feeding experiments primarily was to connect the larval stages with an adult species. Sufficient numbers of cercariae for

other important experiments were not available. The miracidium is undescribed, the length of time necessary for metacercarial development is not known exactly, and the usual location of the adult stage within the host has not been determined satisfactorily.

## SUMMARY

1. A new echinostome cercaria having 27 collar spines and plain tail is described from *Stagnicola emarginata angulata* from the region of Douglas Lake, Michigan.
2. The redia is of the long gut type, having prominent appendages, broken collar, and no pigment.
3. Tadpoles of several species were experimentally infected with the metacercaria which forms a thin-walled cyst in the skin or subcutaneous tissues in the region of the cloaca.
4. The adult stage was obtained by feeding metacercariae which were over three weeks of age to a ferret.
5. The adult worm is identical with a species previously reported from the North American mink (*Mustela vison*), and probably is identical with the European species, *Euparyphium melis* (Schränk, 1788).
6. The North American form is redescribed.

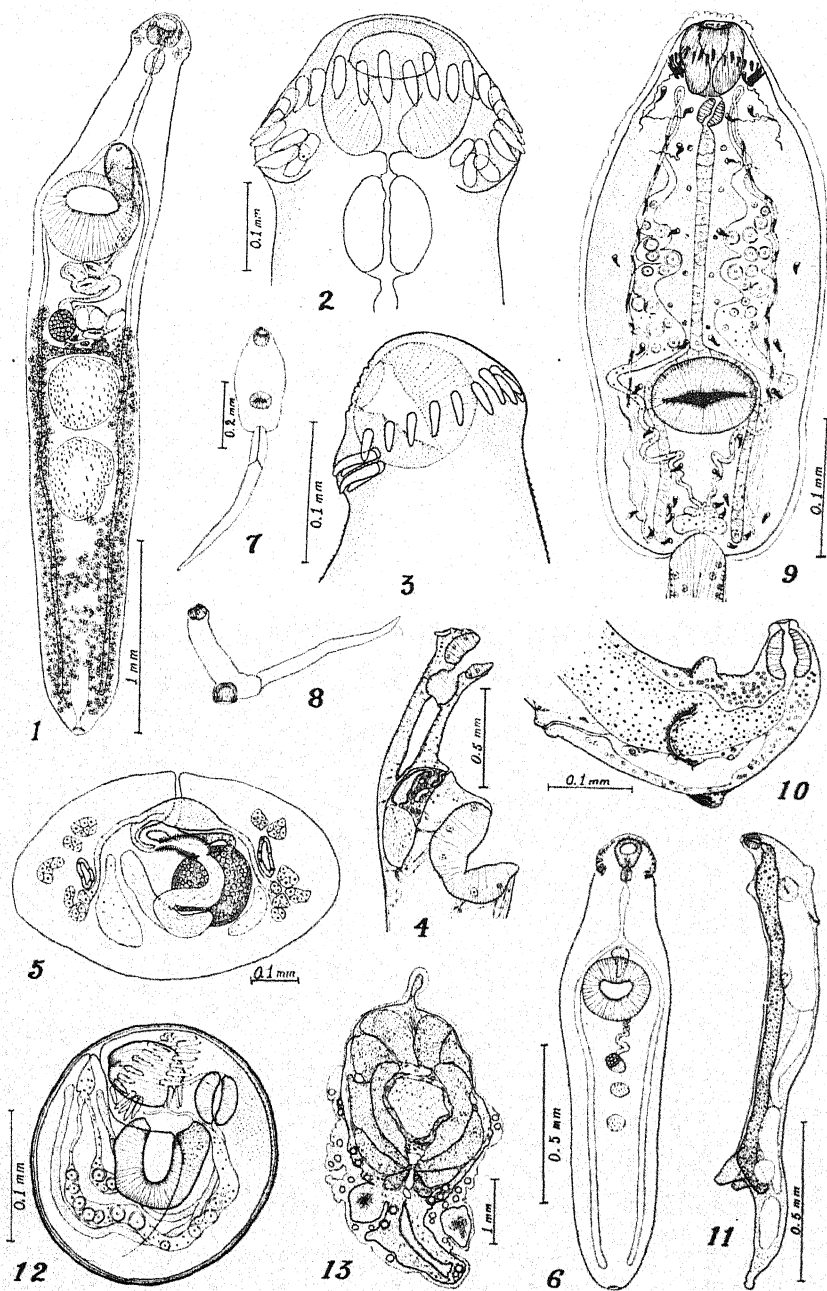
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## EXPLANATION OF PLATE, p. 44

Drawings made with the aid of a camera lucida

- FIG. 1. Adult of average size from a mink. Ventral view.  
FIG. 2. Head crown of the specimen shown in Fig. 1.  
FIG. 3. Head crown of the specimen shown in Fig. 6. Lateral view.  
FIG. 4. Sagittal section through the suckers, cirrus sac, genital pore, esophagus, and pharynx of an adult.  
FIG. 5. Graphic reconstruction of the female genital complex viewed from the posterior end. The dorsal side is above.  
FIG. 6. Young adult from an experimental ferret. Ventral view.  
FIGS. 7 and 8. Cercariae showing typical attitudes of the body and tail.  
FIG. 9. Cercaria. Ventral view of the body moderately extended. (Outlines drawn from an unflattened fixed specimen. Details of the excretory system added.)  
FIG. 10. Redia. Lateral view of the anterior end showing relationship of the mouth, gut, collar, and birthpore.  
FIG. 11. Redia. Lateral view of an average specimen.  
FIG. 12. Metacercaria in cyst. Age 17 to 21 days.  
FIG. 13. Transverse section of an infected tadpole, cut at the level of the cloaca and posterior limb buds. Location and size of metacercariae are shown by the small open circles. About 30 metacercariae appear in this section.

*Euparyphium melis.*

## TRYPANOSOMIASIS IN THE FLORIDA COTTON RAT, *SIGMODON HISPIDUS LITTORALIS*

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The blood of a number of wild Florida cotton rats (*Sigmodon hispidus littoralis*) (1), recently received for experimental purposes by investigators in this laboratory, was examined for the presence of natural infections with trypanosomes, and some of the animals were found to be positive.<sup>1</sup> Since, so far as the writer is aware, no natural trypanosome has previously been described for this species of host, the natural and experimental infection with this form has been studied carefully in some detail. At the same time, the susceptibility of the cotton rat to induced infection with other species of trypanosomes has also been investigated. The observations made during the study provide the data for this report.

### THE NATURAL TRYPANOSOME OF THE FLORIDA COTTON RAT

*Incidence of infection.* The tail blood of 90 cotton rats was examined for trypanosomes promptly on the arrival of these animals by express from the commercial shipper (Zoological Research Supply, Englewood, Florida), who had obtained the animals from the field. Of this number, 18 (20 per cent) were naturally infected with the trypanosome, the parasite being found in the tail blood. The host animals weighed from 50 to 150 grams, small and large animals having about equal incidence of infection. The animals appeared not to suffer from the trypanosome, none succumbing from the effects of either the natural infection or the experimentally induced infections.

*Description of the trypanosome.* In fresh blood examined ( $\times 430$ ) under the cover slip, the trypanosomes moved actively, but made comparatively little headway. They did not readily leave a given microscope field, and often confined their activity to a small zone of the blood fluid from which by their movements they had pushed back the erythrocytes. In blood films treated with Wright's stain, the forms appeared as typical trypanosomes, with a nucleus set only very slightly, if at all, forward of the cell center. A well-developed flagellum and undulating membrane, and a fairly prominent kinetoplast were present. The posterior end of the cell was sharply pointed. No multiplying cells were observed in films of the peripheral blood, although films were prepared only from

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cotton rats infected ten days or more previously. Measurements of 25 trypanosomes with a filar ocular micrometer revealed the following dimensions: length range  $14.8\ \mu$  to  $20.0\ \mu$  with mean length of  $16.9\ \mu$ , and greatest breadth range  $1.6\ \mu$  to  $2.5\ \mu$ , with a mean greatest breadth of  $2.0\ \mu$ . These dimensions for length exclude the free flagellum which ranged from  $2\ \mu$  to  $5\ \mu$ , and those for greatest breadth exclude the undulating membrane. The blood films used for these measurements were prepared by the following technic: They were let dry without special fixation; Wright's stain was applied for 1 minute; an equal amount of tap water was added to the Wright's stain and the mixture let stand on the film for 3 minutes; and the film was washed in tap water. After drying, the film was examined with an oil immersion objective ( $\times 93$ ) and the filar micrometer ocular ( $\times 8$ ).

As yet, only the heart has been examined in section for fixed-tissue stages of the parasite. None was seen in its tissues.

*Cultivation.* Several attempts to cultivate the cotton rat trypanosome in N.N.N. medium were made, but with no success. Inoculated parasites survived up to four days in the test tube, but no evidence of their multiplication was noted. The addition of 0.1 cc of infected cotton rat blood to 5 cc of nutrient broth (a procedure the author has found successful for cultivating *T. cruzi* from the albino rat) also failed to result in the cultivation of the cotton rat trypanosome.

*Normal course of an experimentally induced infection in the cotton rat.* The natural trypanosome infection in the cotton rat can with facility be carried artificially by transfer of infected blood to other cotton rats. In the present work, blood from a heavily infected donor animal was emulsified in nutrient broth and inoculated intraperitoneally. Infections thus induced with this trypanosome generally remained patent for at least one month, following a prepatent period of 6 to 10 days. The peak of the infection occurred about one week after the parasites first appeared in the blood, the parasite count at this time occasionally reaching 25,000 organisms per cmm of tail blood. The data for the course of infection in a representative animal are given in Table 1.

*Natural transmission.* The natural vector of this trypanosome among

TABLE 1.—*The course of an experimentally induced infection with the cotton rat trypanosome in the cotton rat*

Cotton Rat No.	Parasite counts on designated days after infection											
	8	10	12	15	17	20	23	25	28	30	33	35
1	+	+	2T	8T	12T	9T	9T	7T	2T	++	+	0

Key to parasite counts:

0 = no parasites in 50 microscope fields ( $\times 430$ );

+= less than 1 parasite per field;

2+ = from 1 to 5 parasites per field;

2T, 8T = thousands of parasites per cmm of tail blood by hemocytometer.

etc

cotton rats has not been determined. In the pelage of the animals, fleas and lice are often observed for a few days after the cotton rats arrive, but none of a number of such insects on dissection has been found infected with stages of trypanosomes. Mites as well as the fleas have been found in the litter of the shipping cages. The species of the insects and the mite have not been established.

*Experimental inoculation of other hosts.* Rabbits, guinea pigs, and albino rats and mice were inoculated with a broth suspension of the cotton rat trypanosome, to determine the susceptibility of these host species to the parasite. Of the several hosts only the albino rat—and of the rats only young individuals—proved susceptible to demonstrable infection. The susceptible albino rats were nurslings 9 days old when injected. They were positive on examination of tail blood after from 4 to 8 days, and occasional trypanosomes were seen thereafter for about 10 days. The infections were extraordinarily mild in every case, never as many as 1 parasite being seen per 10 microscope fields ( $\times 430$ ). No infection resulted in a second group of nursing rats to which blood from these infected nurslings was transferred (see Table 3).

*Effect of germanin and of normal human serum.* The cotton rat trypanosome was tested for susceptibility to germanin (Bayer 205) and to fresh normal human serum. These materials are without effect upon many species of trypanosomes, including those of the *lewisi* group, although they are highly active in destroying certain of the pathogenic trypanosome, such as *T. equiperdum* and its relatives. The present experiment was carried out upon 5 cotton rats already well infected with their natural trypanosome, to determine whether the cotton rat trypanosome resembled *T. lewisi* or *T. equiperdum* in its susceptibility to germanin and human serum. To 2 of these, 10 mg of germanin was given intraperitoneally and, to a third, 2 cc of fresh normal human serum was injected by the same route. The trypanocidal activity upon *T. equiperdum* of both the drug and the serum was proved by a control test in albino rats infected with *T. equiperdum*. All five of the cotton rats were examined just prior to treatment and again 24, 48, and 72 hours after treatment. In no case was the number of trypanosomes in the cotton rats affected by the treatment, although both substances removed *T. equiperdum* from the peripheral blood of the albino rats within 24 hours.

#### STUDIES ON IMMUNITY WITH THE COTTON RAT TRYPANOSOME

*Resistance to reinfection.* A cotton rat which had recovered just two weeks earlier from a heavy infection with the cotton rat trypanosome (Rat No. 1, Table 1) was, along with a normal cotton rat,<sup>2</sup> reinoculated

<sup>2</sup> Since only field-caught animals were available for this work, one was never certain that new animals used as controls had not recovered from infection prior to arrival in the laboratory. This difficulty was overcome only by using a disproportionately large number of control animals in each experiment.

with the same trypanosome. The recovered cotton rat showed no infection at any time after reinoculation. The control cotton rat showed the parasite on the tenth day after infection, and the parasite count rose subsequently, with its peak about one week later. The results of this test are given, together with the results of a test for the passive transfer of immunity against this parasite, in Table 2.

TABLE 2.—*Specific immunity of the cotton rat after recovery from infection with the cotton rat trypanosome, and the transfer of this immunity in the serum*

Cotton Rat No.	Treatment	Parasite counts on designated days after infection					
		8	10	12	15	17	20
1*	Actively immune by recovery	0	0	0	0	0	0
2†	Passively immune	0	0	0	0	0	0
3	Normal control	0	+	2-	6T	7T	7T

Key to parasite counts: same as Table 1.

\* Same as cotton rat No. 1, Table 1; reinoculated 2 weeks after recovery from initial infection.

† Immunized passively with 1 cc of serum from recovered cotton rat No. 1.

*Passive transfer of acquired immunity in the serum.* A very small normal cotton rat was inoculated together with the 2 cotton rats mentioned in the preceding section with a suspension of cotton rat trypanosomes. This animal was injected at the same time with 1 cc of serum from the cotton rat (No. 1) known to have recovered 2 weeks earlier from infection with the cotton rat trypanosome. The test animal given the specific antiserum remained permanently free of the parasites, although the control animal showed parasites on the tenth day subsequently. The parasite count in the control animal rose thereafter in the usual manner of this infection. It is indicated by this work, the results of which are shown in Table 2, that the immunity of the specifically recovered cotton rat can be passively transferred in the serum to a normal animal.

Thirty-five days after the initial injection of trypanosomes into the passively immunized rat, this animal was reinjected with the cotton rat trypanosome. After a prepatent period of 11 days, the animal became infected and showed the usual course of infection of a normal animal. It is thus shown that the passive immunity in the cotton rat against the cotton rat trypanosome is of the expected comparatively brief duration.

*Immunity of albino rats to *T. lewisi* and of cotton rats to their trypanosome after inoculation with the alternate parasite.* One normal cotton rat was injected with *T. lewisi* and three nursing albino rats were injected with the cotton rat trypanosome. No parasites were seen at any time during 17 days after inoculation in the cotton rat, although a control albino rat injected with the same suspension of *T. lewisi* promptly contracted a heavy infection. This cotton rat was then inoculated with the cotton rat trypanosome. The ensuing infection, which was first

patent twelve days later, lasted 30 days, although its intensity was extraordinarily low and on some days no infection was demonstrable. The three albino rats suffered very mild infections with the cotton rat trypanosomes (as stated also in an earlier section of this paper), and overcame their infections with no difficulty. On inoculation of *T. lewisi* to these albino rats three weeks later, no infection resulted, although control albino rats promptly became infected. From this, it is indicated, as shown in Table 3, that prior inoculation of the albino rat with the cotton

TABLE 3.—*Demonstration of (1) immunity of albino rat to T. lewisi after inoculation with cotton rat trypanosome, and (2) partial immunity of cotton rat to its natural trypanosome after inoculation with T. lewisi*

PART 1: ALBINO RATS

Animal No.	Course of cotton rat trypanosome infection, on designated days							Course of <i>T. lewisi</i> infection, on designated days			
	4	6	8	10	12	14	16	4	6	8	10
1	0	0	+	+	+	+	0	0	0	0	0
2	0	0	+	0	0	0	0	0	0	0	0
3	+	+	0	+	+	+	0	0	0	0	0
4	Controls, not infected with cotton rat trypanosome							3+	4+	4+	3+
5								3+	4+	4+	3+
6								2+	3+	+	0

PART 2: COTTON RATS

Animal No.	Treatment	Course of infection with cotton rat trypanosome, on designated days													
		8	9	10	12	15	17	21	25	27	30	33	35	36	
10	<i>T. lewisi</i> previously inoculated*	0	0	0	+	+	+	+	0	+	+	0	0	0	
11	Control	+	+	6T	15T	23T	13T	11T	7T	7T	8T	2+	+	0	

\* Infection with *T. lewisi* in cotton rat No. 10 was never demonstrated directly in the peripheral blood.

† The heaviest infection with the cotton rat trypanosome occurred on the fifteenth day, when 22 parasites were seen in 50 microscope fields.

Key to counts of cotton rat trypanosome, same as Table 1.

Key to counts of *T. lewisi*:

0 = no parasites in 50 fields of microscope ( $\times 430$ );

+ = less than 1 parasite per field;

2+ = between 1 and 5 parasites per field;

3+ = from 5 to 25 parasites per field;

4+ = over 25 parasites per field.

rat trypanosome, or of the cotton rat with *T. lewisi* protects these animals against infection from the subsequent inoculation of the alternate species of parasite. A close immunological relationship is thus indicated between these two species of parasites.

TAXONOMY OF THE COTTON RAT TRYPANOSOME

In a number of respects the cotton rat trypanosome appears to be distinct from *T. lewisi*: it does not grow easily, if at all, in the usual N.N.N. medium in which *T. lewisi* thrives; its nucleus is not so far forward in the cell as is that of *T. lewisi*; and it does not produce infection in the albino rat comparable in intensity to those with *T. lewisi*. On the other hand, the cotton rat trypanosome appears to be immunologically

similar to *T. lewisi*. Young albino rats on recovery from mild infections with the cotton rat trypanosome are immune to *T. lewisi* and the prior inoculation of cotton rat with *T. lewisi* appears to enhance its resistance to a subsequent infection with the cotton rat trypanosome. This immunological data suggests a close taxonomic relationship between *T. lewisi* and the cotton rat trypanosome, the latter perhaps representing a physiologically distinct strain of the *lewisi* type of trypanosome. Because, however, there are differences in morphology as well as in infectivity from the type species, it seems reasonable to ascribe to the cotton rat trypanosome a new specific name. The name *Trypanosoma sigmodoni* is, therefore, suggested as an appropriate name for the trypanosome of the cotton rat.

*Trypanosoma sigmodoni* n. sp.

**Specific diagnosis:** Protomonadida, Eumonadea, Trypanosomidae. Genus *Trypanosoma*. Characteristics of the genus. This species characteristically presents its nucleus only slightly if at all forward of the cell center, and its somewhat prominent kinetoplast near the pointed posterior end of the cell. There is a well-developed free flagellum, and the usual undulating membrane. Division stages have not as yet been seen in either the blood or the fixed tissues. The trypanosome is small, measurements on 25 parasites being:  $14.8 \mu$ – $20.0 \mu \times 1.6 \mu$ – $2.5 \mu$ ; mean  $16.9 \mu \times 2.0 \mu$  (all measurements exclusive of free flagellum and of undulating membrane). The species produces experimental infections of very low intensity in young albino rats, but does not infect guinea pigs, rabbits, or albino mice.

**Host:** *Sigmodon hispidus littoralis* (Mammalia, Rodentia). Florida cotton rat.

**Habitat:** Blood.

**Locality:** Englewood, Florida.

#### SUSCEPTIBILITY OF THE FLORIDA COTTON RAT TO TRYPANOSOMES OF OTHER HOSTS

Cotton rats were tested for susceptibility to *T. equiperdum*, *T. lewisi*, and *T. duttoni*. The animals were found to be about as susceptible as albino rats to *T. equiperdum*. For example, one cotton rat was dead of the infection, its blood swarming with *T. equiperdum*, 5 days after the parasite was injected (Table 4). *T. lewisi* from the rat and *T. duttoni*

TABLE 4.—Course of infection with *T. equiperdum* in the cotton rat, and in the albino rat

Host	Parasite counts, on designated days after infection				
	1	2	3	4	5
Cotton rat . . . . .	+	+	2+	4+	Dead
Albino rat . . . . .	+	++	3+	4+	Dead

Key to parasite counts:

+ = less than 1 parasite per microscope field ( $\times 430$ );

2+ = from 1 to 5 parasites per field;

3+ = from 5 to 25 parasites per field;

4+ = over 50 parasites per field.

from mice failed to infect the cotton rat, no parasites being found at any time after inoculation, although the appropriate control animals given these parasites promptly became infected.



## DISCUSSION

The cotton rat trypanosome appears to be another species of the *lewisi* group of trypanosomes. It differs from others of the *lewisi* group in several rather significant respects, but resembles the type species immunologically. Perhaps the most significant point of similarity is that recovery from a mild infection with the cotton rat trypanosome will completely protect a young albino rat against *T. lewisi*. Only nursing albino rats have proved susceptible to infection with the cotton rat trypanosome; it seems possible that nursing cotton rats (which were not available for the research) might likewise have contracted demonstrable infections with *T. lewisi*.

There is some interest among investigators at present as to whether the cotton rat is immunologically more like the albino rat or the guinea pig. It has been found to be more like the guinea pig in its susceptibility to diphtheria toxin (2), and tuberculosis (3), but like the rat in its refractoriness to anaphylactic shock (4). The high susceptibility and early death of the cotton rat from the pathogenic trypanosome *T. equiperdum* ranges this host with the rat rather than with the guinea pig with respect to its capacity for resistance against this trypanosome infection.

## SUMMARY

A trypanosome found naturally in the peripheral blood of the Florida cotton rat, *Sigmodon hispidus littoralis*, is described. The name *Trypanosoma sigmodoni* is suggested for the parasite. The organism has been found in the blood of 18 (20%) of 90 Florida cotton rats examined. Infections can be readily induced artificially in normal cotton rats by inoculating them with the blood from an infected animal. Following a prepatent period of 6 to 10 days after the injection of infective material, the parasites are found in the peripheral blood for about one month. Infections generally do not rise above an intensity of 25,000 organisms per cubic millimeter of blood, and none of the animals studied has died of its infection. Nursing albino rats are susceptible to very mild infections with the cotton rat trypanosome.

On recovery from infection with this trypanosome, the cotton rat is immune to reinfection, and its serum inoculated to a normal cotton rat protects the normal animal from infection. The trypanosome appears to be somewhat closely related immunologically with *T. lewisi*, since young albino rats which have recovered from an experimentally induced infection with the cotton rat trypanosome are protected against *T. lewisi*. Likewise, a cotton rat previously inoculated with *T. lewisi* acquires a partial immunity against the cotton rat trypanosome.

The cotton rat is highly susceptible to *T. equiperdum*, succumbing to infection with this species in about 5 days. The cotton rat resists infection with *T. lewisi* and *T. duttoni*.

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## THE PENETRATION OF RADIOACTIVE PHOSPHORUS INTO ENCYSTED *TRICHINELLA* LARVAE

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It is well-known that *Trichinella spiralis* larvae can live for an extraordinarily long time encysted in the muscles of a host. There are many reports in the literature describing larvae which have been found alive in human muscle, for from 5 to 30 years after the disease was thought to have been contracted (1, 2, 3, etc). In the authors' experience, numerous living larvae were found in a muscle removed at biopsy from a man who had had a very severe attack of trichiniasis seven and one-half years previously. This man had had the cause of his illness explained to him and had been so afraid of contracting the disease a second time that he claimed that he had not eaten meat of any kind since. It seems probable, therefore, that the larvae found in this case must have been present since the original illness.

The generally accepted view, as expressed many years ago by Staubli (4), is that, after encystment, *Trichinella* larvae enter a latent stage in which they are walled-off from the tissues of the host. It is true that the larvae do not grow or develop while encysted, but it is well-known that they are capable of movement within their cysts. Stannard, McCoy and Latchford (5) showed that larvae digested free from their cysts exhibit both an aerobic and an anaerobic metabolism when kept in Tyrode's solution at 37° C. It is not known whether encysted *Trichinella* larvae live on food materials stored within their own bodies, or whether they absorb nourishment through the cyst wall from the host. In view of the long periods of time during which they remain viable in the encysted stage, the latter seems the more probable. The present preliminary experiments are designed as a test of the permeability of the cyst wall.

Within the past few years, the production of radioactive isotopes of such elements as sodium, potassium, phosphorus, iron, etc, has afforded the means for tracing the distribution of these substances in the bodies of experimental animals. This method of study, of course, is equally applicable to parasites which may be present in these animals. In the present experiments, tests were made of the ability of encysted *Trichinella*

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<sup>1</sup> This investigation was suggested by Dr. George Packer Berry, Department of Bacteriology, and was aided by the helpful advice of Dr. William F. Bale, Department of Radiology. The work was assisted in part by a grant-in-aid from the Rockefeller Foundation.

larvae to absorb radioactive phosphate ions. Phosphorus was selected as the test substance because a source of the radioactive element was readily available, and because the half-life of radioactive phosphorus is relatively long (14.5 days), thus convenient for experiments lasting for several days or more.

#### MATERIALS AND METHODS

The tests were made upon laboratory-bred albino rats of Wistar stock which had been heavily infected with *Trichinella spiralis* from 8 to 10 months previously. After this period of time, the larvae were enclosed in thick-walled cysts of hyalinized connective tissue characteristic of long-standing muscle infection. (Fig. 1.) No calcification, however,

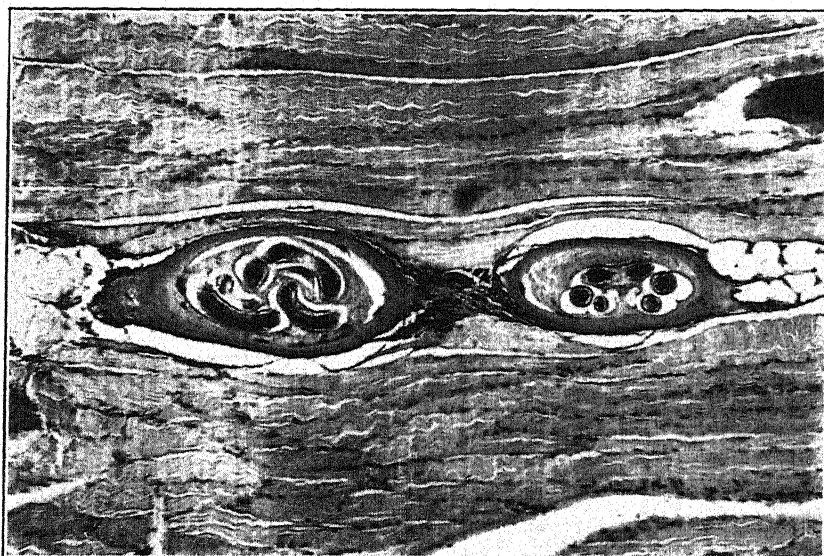


FIG. 1. Encysted *Trichinella spiralis* larvae in the muscle tissue of a rat killed 8 months after infection (magnification  $\times 100$ ).

was observed. It was considered that cysts of this age and degree of development would offer a fair test of the permeability of *Trichinella* cysts in general.

The rats were fed by stomach tube 1-cc amounts of a solution containing radioactive phosphorus in the form of basic sodium phosphate. They were killed thereafter at intervals varying from 2 hours to 6 days. After removing 3-gm samples of muscle for analysis, the carcasses were digested for 3 hours at  $37^{\circ}\text{C}$  with a solution of 0.7 per cent pepsin and 0.5 per cent hydrochloric acid. The larvae freed from their cysts were collected by sedimentation, washed free from debris, desiccated and weighed.

The amount of radioactive phosphorus present in the larvae was de-

terminated by the use of a Geiger-Müller counting apparatus according to the method described by Bale, Haven and LeFevre (6). The amount of radioactive phosphorus is calculated in terms of the percentage of the original dose fed to the rats which was determined per gram of phosphorus present in the larvae. This figure is called the "specific radioactivity" of the larvae and enables a comparison to be made with the amount of radioactive phosphorus present in the muscles. To calculate this figure it was, of course, necessary to determine the amount of phosphorus in each sample of larvae and of muscle.<sup>2</sup>

In the procedure followed for recovering the larvae from the infected muscle, it was possible that the larvae might take up radioactive phosphorus after being digested free from their cysts. In order to test this possibility, the carcasses of uninfected rats which had been fed radioactive phosphorus were ground up and digested according to the same procedure used for the experimental animals. At the time digestion was begun, however, normal larvae, recovered from rats *not* fed radioactive phosphorus, were added to the digestion fluid. When tested subsequently, it was found that these larvae did not contain a measurable amount of radioactive phosphorus. Two tests of this nature were performed in each of the three experiments. It seems reasonable to conclude, therefore, that the radioactive phosphorus detected in the larvae must have been absorbed while they were still within their cysts.

#### EXPERIMENTAL

*Experiment 1.* Eight female rats infected with *Trichinella* 10 months previously were used. They were killed in pairs at intervals of 1, 2, 4 and 6 days after feeding radioactive phosphorus. The results are tabulated in Table 1. A significant amount of radioactive phosphorus was detected in the larvae from rats killed after 24 hours, and the greatest relative amount was present in the larvae from those killed after 4 days.

*Experiment 2.* Six male rats infected for 8 months were killed in pairs at intervals of 12, 24 and 48 hours after feeding radioactive phosphorus. The results, which are also tabulated in Table 1, support those found in the first experiment. The larvae recovered from one of the rats (No. 9) killed after 12 hours showed an abnormally large amount of radioactive phosphorus in comparison with that found in the larvae recovered from the other animals. This figure, therefore, is omitted from the averages. The larvae from the other rat killed after 12 hours contained almost as much radioactive phosphorus as the average amount found in the larvae from the two rats killed after 24 hours.

*Experiment 3.* Ten male rats infected 8 months previously were killed in pairs at intervals of 2, 4, 8, 12 and 24 hours after feeding radio-

<sup>2</sup> The writers are indebted to Dr. Richard S. Manly and Miss Sylvia Levy, Department of Biochemistry, for performing these analyses.



TABLE 1.—Amount of radioactive phosphorus present in *Trichinella* larvae and the muscles from rats killed at intervals after feeding radioactive phosphorus

Rat No.	Interval between feeding P and death	Dry weight of larvae recovered (in mgm)	Per cent of phosphorus in larvae	Specific radio-activity of larvae*	Wet weight of muscle sample (in gm.)	Per cent of phosphorus in muscle	Specific radio-activity of muscle*
<i>Experiment 1.—Female rats infected for 10 months</i>							
1	24 hr.	115	0.67	11.7	3.156	0.18	5.6
2	24 "	80	0.69	12.3	3.107	0.18	5.5
3	48 "	68	0.69	26.4	2.682	0.18	6.2
4	48 "	205	0.71	13.5	2.935	0.18	5.7
5	4 days	130	0.40	24.9	2.840	0.20	7.6
6	4 "	55	0.84	24.6	2.865	0.21	6.9
7	6 "	100	0.64	18.5	3.050	0.18	4.8
8	6 "	95	0.70	20.9	3.122	0.18	5.8
<i>Experiment 2.—Male rats infected for 8 months</i>							
9	12 hr.	102	0.76	70.6†	2.567	0.21	19.4
10	12 "	94	0.67	14.3	2.632	0.23	28.1
11	24 "	90	0.57	19.3	2.582	0.22	21.3
12	24 "	115	0.78	11.5	2.705	0.23	13.4
13	48 "	87	0.54	20.3	2.413	0.22‡	16.7
14	48 "	76	0.67	11.5	3.264	0.22	16.7
<i>Experiment 3.—Male rats infected for 8 months</i>							
15	2 hr.	153	0.74	0.86	2.232	0.22	7.22
16	2 "	215	0.66	1.08	3.290	0.21	6.04
17	4 "	174	0.80	2.71	3.038	0.19	7.30
18	4 "	184	0.70	3.06	4.159	0.18	7.36
19	8 "	169	0.74	2.68	3.816	0.22	9.44
20	8 "	195	0.71	3.92	3.094	0.19	6.59
21	12 "	165	0.71	6.44	3.848	0.23	9.38
22	12 "	251	0.76	5.45	2.735	0.23	8.83
23	24 "	126	0.45	4.99	3.045	0.20	8.70
24	24 "	239	0.84	19.20	2.423	0.21	11.60

\* "Specific radioactivity" is the amount of radioactive phosphorus present per gram of phosphorus expressed as the percentage of the original dose fed.

† This figure not included in averages.

‡ Sample lost.

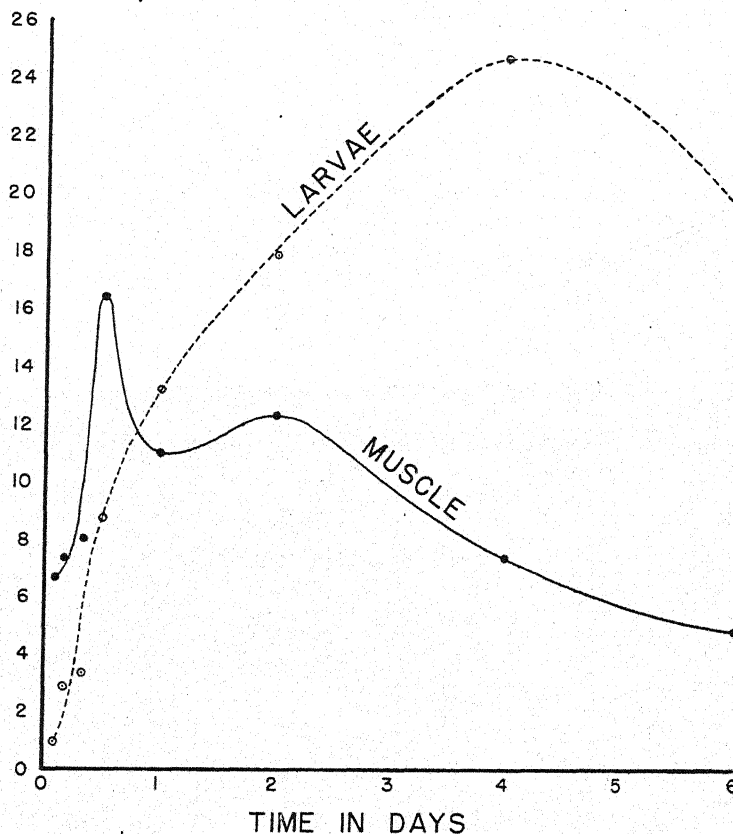
active phosphorus. The results are also shown in Table 1. The amount of radioactive phosphorus found in the larvae recovered from the rats killed after 2 hours was about the minimum that could be detected by the method employed. Increasing amounts were found in the larvae from the rats killed after from 4 to 24 hours.

#### DISCUSSION

The "specific radioactivity" of the larvae recovered from pairs of rats killed at the same time showed considerable variation in all three experiments. Experimental error in desiccating and weighing the larvae, in analyzing their phosphorus content and in making the counts probably accounts for some of this variation. In addition, there appears to be an inherent variability in the different lots of rats that were used. For instance, the "specific radioactivity" of the muscles of the rats in Exper. 1 was much less than that measured in the other two experiments. The fact that these rats were of a different sex and were two months older than the rats in the other experiments may account for this difference. It should be mentioned, also, that the three experiments were performed at different seasons of the year.

The figures for the "specific radioactivity" of the larvae and of the muscles from rats killed at the same intervals after feeding radioactive phosphorus were averaged for the three experiments and plotted (Fig. 2). The individual points represent the average results in from 2 to 6

SPECIFIC  
RADIOACTIVITY  
(% OF FED DOSE  
PER GRAM OF P)



(POINTS ARE AVERAGES OF RESULTS IN FROM 2 TO 6 ANIMALS)

FIG. 2. The relative amount of radioactive phosphorus found in *Trichinella* larvae and muscles from rats killed at intervals of from 2 hours to 6 days after feeding. The individual points are the averages of the results in from 2 to 6 animals. The curves are drawn free-hand and fitted by inspection.

animals. The curves were drawn free-hand and fitted by inspection. Caution must be used in interpreting these curves because of the variability of the material on which they are based. It appears, however, that the rate of absorption and retention of radioactive phosphorus followed a

course different from that for the muscles. The radioactive phosphorus was taken up more rapidly by the muscles and was also lost more rapidly.

The data available in the present experiments are not sufficient to indicate whether the absorption of radioactive phosphorus by the larvae represents an actual metabolic exchange or merely a diffusion of phosphate ions into the larvae. The rapidity of absorption by the encysted larvae, however, might indicate that they are undergoing active metabolism. In any event, it is established that the fully developed cyst wall surrounding *Trichinella* larvae in muscle tissue is permeable to phosphate ions.

#### SUMMARY

In three experiments, 28 rats, heavily infected from 8 to 10 months previously with *Trichinella spiralis*, were fed radioactive phosphorus (as basic sodium phosphate) and killed at intervals beginning 2 hours later. Radioactive phosphorus was detected in the larvae as early as 2 hours after feeding and the amount increased rapidly during the first 24 hours. The maximal content was reached on the fourth day, after which there was a gradual decline. The absorption and loss of radioactive phosphorus by the larvae follow a course different from that for the hosts' muscles. The experiments demonstrate that a ready exchange of phosphate ions may take place through the cyst wall surrounding *Trichinella* larvae in the muscles of rats, and imply that the larvae may be undergoing active metabolism during the encysted stage.

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## AMPHIBIAN NEMATODES FROM THE GASPÉ PENINSULA AND VICINITY

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Through the courtesy of Dr. J. S. Rankin, Jr., of Amherst College, a number of nematodes from amphibian hosts, collected during a trip to the Gaspé Peninsula in June, 1938, were sent to the writer for identification. A number of new locality records for several known species of nematodes are hereby added to the literature, as well as the description of a new species of the genus *Spironoura* Leidy, 1856.

*Rana pipiens* (5 specimens from Matapedia, Gaspé Peninsula) is a host for *Cosmocercoides dukae* (Holl, 1928) Wilkie, 1930.

*Rana clamitans* (8 specimens from near Matapedia, Gaspé Peninsula) is a host for *Foleyella ranae* Walton, 1929, and for an *Oxysomatium* species. Only immature females of the latter were present, so no species designation is attempted.

*Rana clamitans* (6 specimens from Madelene, Gaspé Peninsula) is a host for *Cosmocercoides dukae* (Holl, 1928) Wilkie, 1930.

*Rana clamitans* (2 specimens from Chatham, New Brunswick) is a host for *Foleyella americana* Walton, 1929.

*Rana clamitans* (2 specimens from Machias, Maine) is a host for *Foleyella ranae* Walton, 1929, *Oswaldocrusia leidyi* Travassos, 1917, and *Spironoura rankini* n. sp.

*Rana palustris* (1 specimen from Machias, Maine) is a host for *Oswaldocrusia leidyi* Travassos, 1917.

*Bufo americanus* (1 specimen from Machias, Maine) is a host for *Oswaldocrusia pipiens* Walton, 1929.

*Spironoura rankini* n. sp.

(Fig. 1)

*Description:* Based on male material only. With the characters of the genus: head with three well-developed lips bearing two outer and two inner papillae; oral cavity supported by a cuticular ring; pharynx muscular; esophagus with terminal bulb and a pre-bulbar swelling; lateral fields wide; lateral and caudal alae absent; pre-cloacal musculature well developed; spicules sickle-shaped and alate; accessory piece present. Length, 20.25 mm; greatest width, 0.375 mm; length of pharynx, 0.112 mm; length of esophagus, 1.45 mm; pre-bulbar swelling, 0.225 mm in length and 0.075 mm in width; bulb spherical, 0.185 mm in diameter; head-nerve ring distance, 0.66 mm; head-excretory pore distance, 1.5 mm; cloaca-tail distance, 0.375 mm; spicule length, 1.65 mm, laterally compressed and with narrow unilateral alae; accessory piece length, 0.112 mm; 3 pairs of pre-cloacal papillae, 4 pairs of para-cloacal papillae, and 3 pairs of post-cloacal papillae, in addition to the unpaired median papillus on the anterior margin of the cloaca.

*Host:* *Rana clamitans* (collected June 22, 1938, at Machias, Maine).

*Habitat:* Intestine.

*Type specimens:* Co-types deposited in U. S. Nat. Mus. Helm. Coll.

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\* Contribution from the Biological Laboratories of Knox College, No. 67.

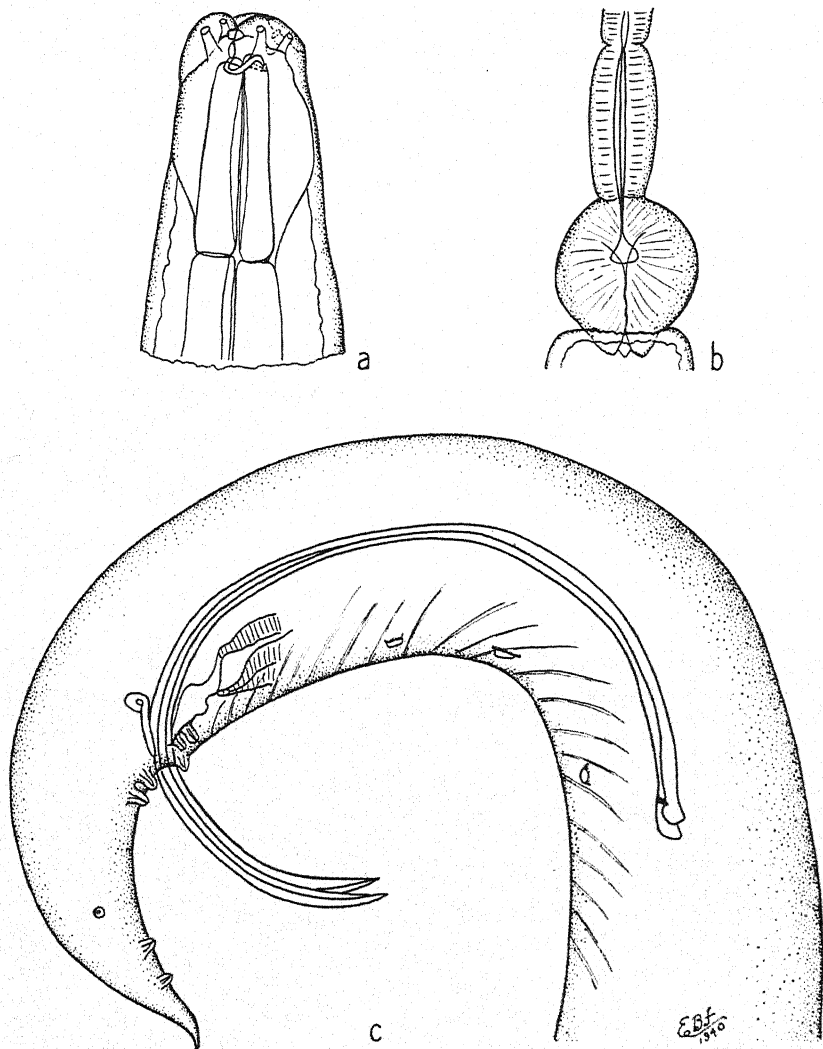


FIG. 1. *Spironoura rankini*. a. Head end of male.  
b. Esophageal bulb from male.  
c. Lateral view of tail of male.

The present species, while much larger than the other species reported from North American amphibia, most nearly resembles *Spironoura elongata* (Baird, 1858) Walton, 1932, from *Ambystoma tigrinum*, and *S. pretiosa* Ingles, 1936, from *Rana pretiosa*, but differs in the relative proportions of the structures at the anterior end, in the sizes of the spicules and accessory piece, and in the number and arrangement of the caudal papillae. *S. rankini* is more than twice the length of any of the species except *S. elongata* and *S. pretiosa*, and is a third longer than adult males of these two species. The specific name is in honor of the collector of this material.



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TWO NEW MESOZOA FROM CALIFORNIA, *DICYEMENNEA CALIFORNICA* AND *DICYEMENNEA BREVICEPHALA* (DICYEMIDAE)\*

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Our knowledge of the DICYEMIDAE (MESOZOA) is almost entirely embodied in several excellent works on the European species. Those of the Pacific Ocean have been neglected and are unknown except for the work of Dr. Wm. Morton Wheeler (1897), who named three species found at San Diego, California, but did not describe or figure them, and that of Nouvel and Nakao (1938) who described a few species of *Dicyema* from Japan. During the summers of 1937 and 1939 and in December of 1938 I examined the kidneys of several octopi from the littoral zone in the Laguna Beach area and of one from the San Francisco Bay area. All the octopi examined except those a centimeter or less in length, were richly infected. The two most abundant species are herein described.

*Dicyemennea californica*, n. sp.

(Figs. 1, 2, 3, 6, 8)

*Specific diagnosis:* Body attenuate, vermiform individuals mostly 1.7 to 3.0 mm long. Calotte enneamerous (4 propolar cells, 5 metapolar cells), conical, forming about a third to over half the cephalic swelling in the adult. Parapolar cells shield shaped forming from about half to two thirds of the cephalic swelling. Total number of peripheral cells 36 or less (primary rhombogenes with 31). Uropolar cells usually swollen and vacuolated distally. Cilia nearly uniform, slightly denser on the calotte than over the trunk. Infusoriform individuals 38 to 45 micra in length, rounded posteriorly.

Nematogenes usually 25 to 50 micra wide, broadest at the level of the parapolar cells. Peripheral cells 36 in number. Calotte conical, orthotropal, made up of four propolar cells (two dorsal and two ventral), and five metapolar cells (one dorsal, two dorso-lateral, two subventral). Parapolar cells shield shaped, lateral, their broad anterior ends encircling the axial cell just behind the calotte.

Transitional stages from nematogene to rhombogene with greatly increased number of agametes in the axial cell, which is broader than in the nematogene, and often contains simultaneously more or less advanced vermiform larvae and one or more young infusorigenes. Vermiform larvae in such cases mostly with the reduced number of cells (31).

Rhombogenes noticeably broader and more robust in appearance than the nematogenes. Primary rhombogenes with 31 instead of 36 peripheral cells, the nuclei of the trunk cells often enlarged and sometimes constricted in two giving rise to accessory nuclei in some of the peripheral cells. Accessory nuclei also frequently occur in the axial cell in this stage.

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\* The writer wishes to express his appreciation to Dr. W. A. Hilton of Pomona College for the use of the facilities of the Pomona College Marine Laboratory at Laguna Beach, to Dr. S. S. Berry of Redlands University for determining the host species of *D. brevicephala*, and to Dr. S. F. Light and Dr. Harold Kirby, Jr. of the University of California for aid and advice in the presentation of this material.

Infusoriform individuals 38 to 45 micra in length when fully developed, rounded posteriorly.

*Host:* *Octopus bimaculatus* Verrill.

*Distribution:* Southern California coast, taken in the littoral zone at Laguna Beach, Emerald Bay and Balboa Bay, Orange Co., Calif.

*Type specimens:* U. S. Nat. Mus. No. 22682, paratype No. 22683, other paratypes in Univ. Calif. Coll. and of author.

*Dicyemenea brevicephala*, n. sp.

(Figs. 4, 5, 7, 9, 10)

*Specific diagnosis:* (There has been less material of this species available to me than of *D. californica*, all of it being from one octopus taken at Marine View Rocks, San Mateo Co., Calif., early in June 1939. Practically all the dicyemids were mature rhombogenes.)

Medium sized to small dicyemids, mostly 1 to 2 mm long. Total number of peripheral cells 27, this probably the number for primary rhombogenes. Calotte enneamerous, short, wide, appearing in many cases as a flattened disc at the truncate ends of the elongated parapolar cells. Metapolar cells usually from two to five times as large as the propolar cells, short, frequently wider than long. The cilia of the calotte shorter and more closely set than those of the trunk cells. Parapolar cells usually very elongate, forming most of the cephalic swelling, generally 100 to 150 micra in length. Uropolar cells not often swollen and vacuolated as in *D. californica*, quite similar in appearance to the other trunk cells. Older rhombogenes frequently much swollen anteriorly, the cells of the calotte becoming enlarged and displaced obscuring their typical arrangement, anterior end of axial cell also swollen in these cases. Infusoriform individuals 28 to 35 micra in length when fully developed, rounded posteriorly.

*Host:* *Octopus apollyon* (Berry).

*Distribution:* Known only from Marine View Rocks, San Mateo Co., Calif.

*Type specimens:* U. S. Nat. Mus. No. 22680, paratypes No. 22681, other paratypes in the Univ. Calif. Coll. and of author.

The two species here described differ in several respects from other known *Dicyemenea*. *D. lameerei* Nouvel, *D. eledones* Wag. and *D. gracile* Wag. each possess only 23 peripheral cells. *D. eledones* and *D. gracile* are both larger species, ranging from 4 to 7 mm in length, and their parapolar cells do not contribute much to the cephalic enlargement. *D. lameerei* tends to be smaller and more massive, ranging from 1 to 1.5 mm in length, and is further distinguished by the constant presence of fine crescentic pointed crystalloid needles in the cytoplasm of the peripheral cells and by the fact that the infusoriform is pointed posteriorly and has only one nucleus and one germ cell in each of the cells of the urn. The host of *D. lameerei* is *Octopus vulgaris* Lam., those of *D. eledones*, *Eledone moschata* Leach and *E. aldrovandi* D. Ch., and that of *D. gracile*, *Sepia officinalis* L.

*Dicyemenea mulleri* Clap. was described by Claperède from specimens taken from *Eledone cirrosa* Lk. along the coast of Norway. As neither the total number of peripheral cells nor the number of cells in the calotte was determined its status remains in doubt. *D. whitmanii* Wheeler is a nomen nudum, no description or figures of it having been published.

Transitional stages between the nematogene and rhombogene phases of *D. californica* were found. These show a great multiplication of the agametes. Several individuals contained advanced embryos of the vermiform stages and one or more young infusorigenes and in some cases even very young infusoriform embryos in the axial cell simultaneously. The young infusorigenes already have the small scattered chromatic bodies always found associated with the infusorigenes of dicyemids. These have been interpreted as sperm by Wheeler (1897), Hartmann (1906), Lameere (1918) and Nouvel (1933), and as irregularly scattered chromosomes resulting from abnormal divisions of the cells of degenerating pycnotic infusorigenes near the end of the rhombogene phase by Gersch (1938). In my material they are found associated both with the very young infusorigenes in transforming individuals before any infusoriforms have been formed and with the older infusorigenes. The cells of the younger infusorigenes do not show evidence of pycnosis, and the chromatic bodies are larger than the individual chromosomes seen in the cells and are quite constant in shape. I have as yet, however, not been able to determine definitely their exact mode of origin and am not fully satisfied that they are sperm.

Nouvel (1938) called attention to a very curious point in the embryology of the vermiform individuals in *Dicyemennea lameerei* and *D. eledones*, in which the first germ cell of the developing larva upon division gives rise to two cells, one of which becomes the mother cell of all the future germ cells or axoblasts and the other of which takes a position at the anterior end of the axial cell, then invades the dorsal metapolar cell of the embryo where it remains, finally degenerating and disappearing completely after the larva escapes from its parent.

This phenomenon is very clearly shown in my material in both species and in both the nematogene larvae and those destined to become primary rhombogenes. It is probably general in *Dicyemennea* but has not been noted in any species of the related genus *Dicyema* the embryology of which has been carefully investigated by several authors.

#### SUMMARY

Two species of *Dicyemennea* from the kidneys of octopi of the littoral zone of the California coast are described. They differ from other known *Dicyemennea* in cell number, size, composition of the cephalic swelling and other details.

Some evidence against Gersch's theory of the origin of the sperm-like chromatic bodies associated with the infusorigene is presented.

The phenomenon described by Nouvel wherein one of the cells resulting from the first division of the primary germ cell in the vermiform embryo invades the dorsal metapolar cell and degenerates there has been confirmed. It is probably general in *Dicyemennea*.



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## EXPLANATION OF PLATES

All figures drawn with the aid of camera lucida

## PLATE I

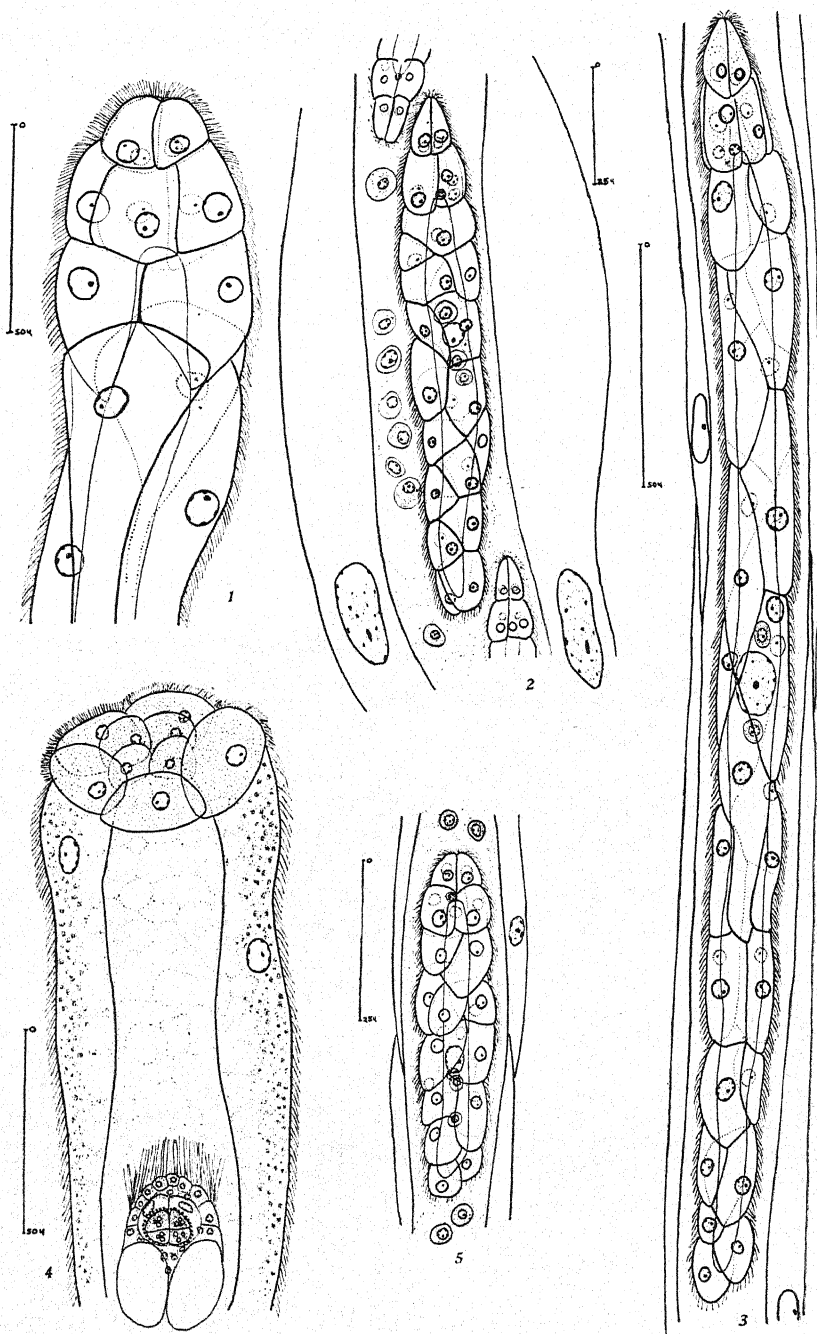
FIG. 1. Calotte of *Dicyemenea californica*, late nematogene. (Bouin—Jansens haematoxylin.)

FIG. 2. Primary rhombogene larva in axial cell of an individual transitional between the nematogene and rhombogene phases, showing the reduced number of peripheral cells (31) and the degenerating germ cell in the dorsal metapolar cell just anterior to the end of the axial cell. (Bouin—Ehrlich's haematoxylin.)

FIG. 3. Nematogene larva in the axial cell of a primary nematogene, showing the 36 peripheral cells and the degenerating anterior germ cell. (Bouin—Ehrlich's haematoxylin.)

FIG. 4. Calotte of *Dicyemenea brevicephala*, mature rhombogene. (Bouin—borax carmine, indulin.)

FIG. 5. Vermiform larva of *D. brevicephala* (probably a primary rhombogene) showing 27 peripheral cells and the degenerating anterior germ cell. (Bouin—borax carmine, indulin.)



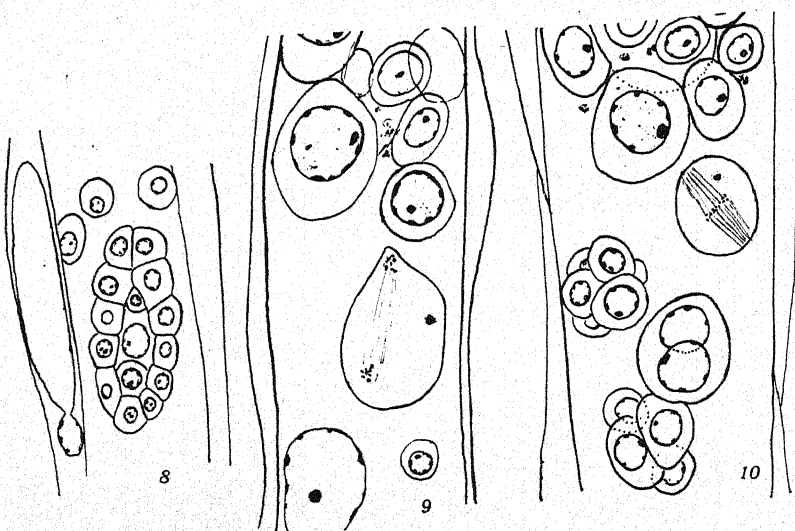
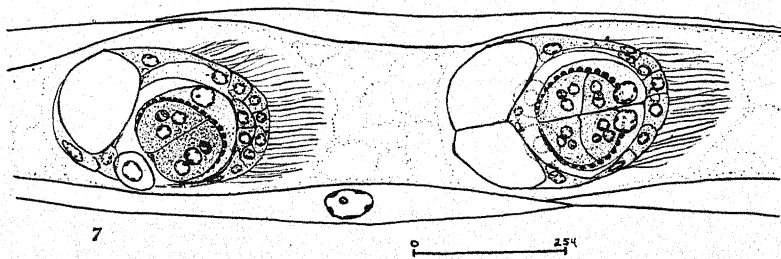
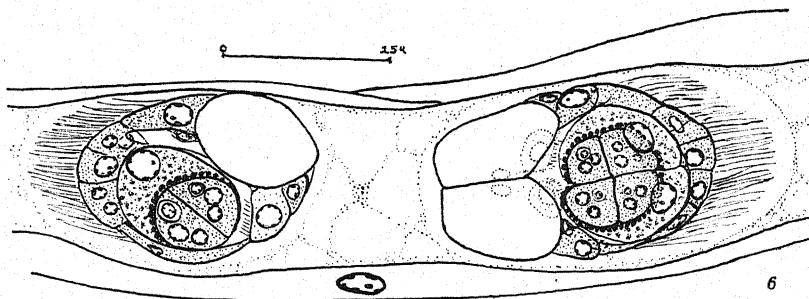
## PLATE II

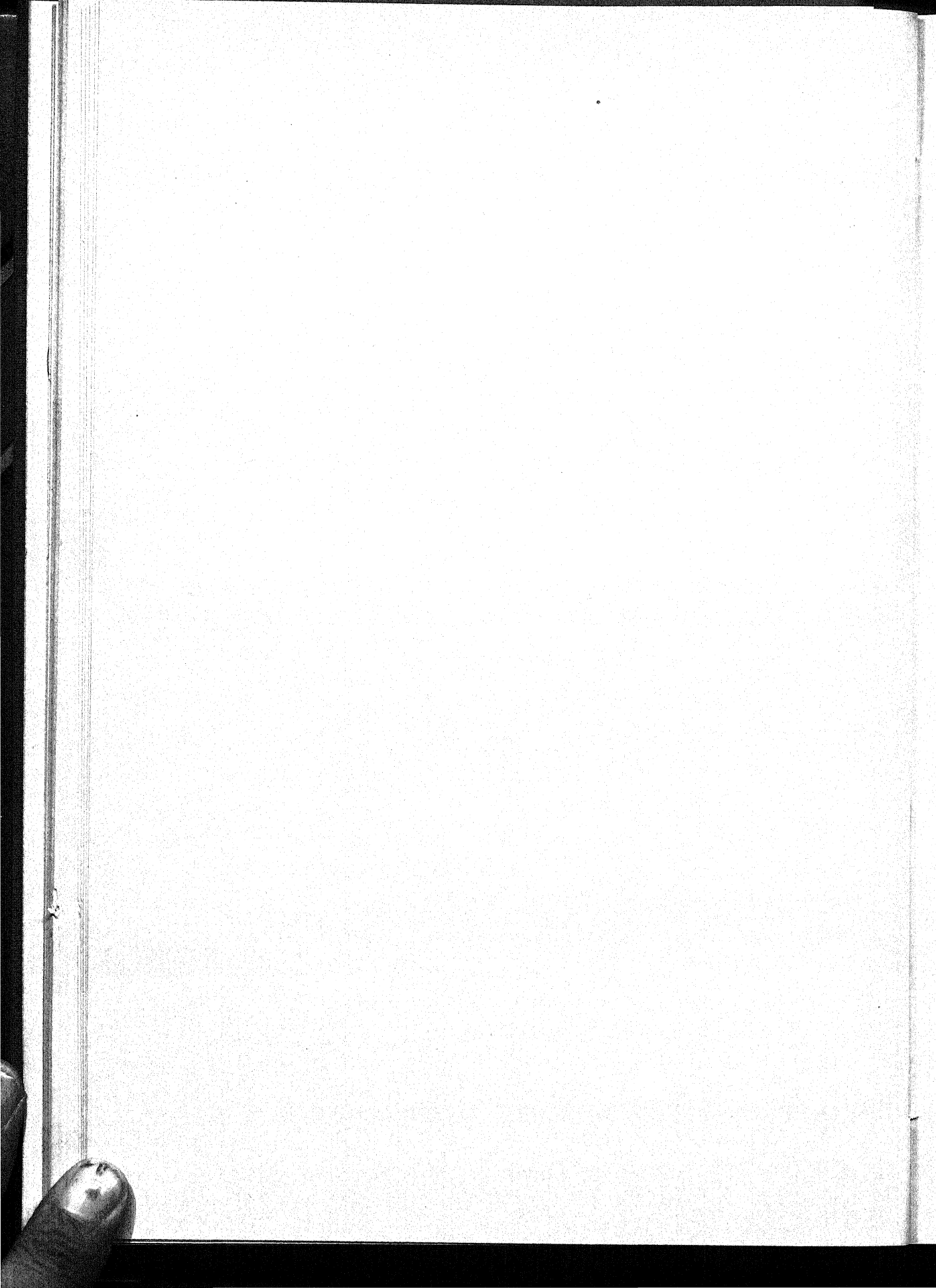
FIG. 6. Infusoriform of *D. californica* parasagittal and frontal optical sections. (Bouin—Ehrlich's haematoxylin.)

FIG. 7. Infusoriform of *D. brevicephala*, same views. I think there should be cilia in the cavity of the urn here too but was unable to see them clearly in this case. (Bouin—Jansens haematoxylin.)

FIG. 8. Very young vermiform of *D. californica*, about 22 cell stage, in optical section, showing the axial cell and the two cells resulting from the first division of the primary germ cell, one, posterior and larger, the mother cell of all the future axoblasts; the other, anterior and smaller, destined to invade the dorsal metapolar cell and degenerate there. (Bouin—Ehrlich's haematoxylin.)

FIGS. 9 and 10. Portions of infusorigenes and division of the "egg" cells in *D. brevicephala*, showing conditions cited by several authors as evidence of sperm penetration and polar body formation. In Fig. 10 there is also shown an "egg" cell with two nuclei which are regarded by most authors as the male and female pronuclei before the first cleavage but by Gersch (1938) as merely two nuclei resulting from failure of the cytoplasm to divide following nuclear division. These stages are relatively infrequent in my preparations but where they occur they are almost always in the same order passing from the infusorigene outwards. (Bouin—Jansens haematoxylin.)







## THE INCIDENCE OF INTESTINAL PARASITES IN A SELECTED GROUP AT A MENTAL HOSPITAL\*

MARTIN D. YOUNG AND COYT HAM

U. S. Public Health Service and the South Carolina State Hospital

This report is of a survey conducted to determine the incidence of parasites in a selected group of patients. The cases examined were all white women of varying ages but about the same mental level, untidy in their habits, and confined in a separate building with semiprivate recreation grounds.

Strict hygienic measures have been employed during the residence of the patients. The building is kept in good repair, paying particular attention to the sealing of all small openings where filth and contamination might collect. The floors are of glazed tile. The recreation grounds consist of two separate yards; these are limed, worked over and resodded with new grass and each yard is unoccupied for alternate six months intervals. Even with such measures, the problem of parasitism is still acute.

### MATERIALS AND METHODS

Examinations were made of freshly obtained fecal specimens which were collected in one-half pint cardboard containers. Two specimens were examined from all patients, but some, especially those found to be infected with *Balantidium coli*, were repeatedly examined. Simple saline and saline-iodine smear preparations were used. No technique for concentration of parasites was employed. It is recognized that concentration techniques will reveal infections not found by the simple smear method and therefore the incidence should be considered as a minimum for the group. Undoubtedly many low grade infections were not detected.

Following the stool examinations, the patients were given anthelmintics. It should be borne in mind that these patients have been treated in the past at regular intervals. At the time of each periodic medication numerous parasites were eliminated.

### OBSERVATIONS

One hundred forty-two patients were examined and parasites were found in 128 or 90 per cent. One hundred seventeen (82 per cent) harbored helminths and 92 (65 per cent) were infected with protozoa.

The number of species of parasites harbored by the patients were as follows: 14 patients had no species of parasites; 11 had one; 22 had 2;

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\* Contribution from the Williams Malaria Research Laboratory, Malaria Investigations, National Institute of Health and the South Carolina State Hospital, Columbia, S. C.

35 had 3; 29 had 4; 15 had 5; 10 had 6; 5 had 7; and one had 8 species. This gives a total of 454 specific infections in the 142 patients or an average of 3.2 species per person.

Table 1 shows the distribution of parasites by species and grouped according to the length of residence of the patients in the hospital.

TABLE 1.—Distribution of parasites according to the length of residence of the patients in the hospital

Years in Hospital	Less than one	1-5	6-10	11-15	16-20	21-25	26-30	31+	Total	
No. examined	6	44	24	32	17	7	8	4	142	
Infected	%	%	%	%	%	%	%	%	No.	%
<i>Strongyloides stercoralis</i>	17	39	41	41	50	29	38	50	56	39
Hookworm	17	66	71	59	77	43	100	100	94	66
<i>Trichuris trichiura</i>	50	73	75	78	94	57	88	100	111	78
<i>Ascaris lumbricoides</i>	17	7	13	9	0	14	13	50	14	10
<i>Endamoeba histolytica</i>	0	3	4	7	0	14	0	0	5	4
<i>Endamoeba coli</i>	33	45	57	50	50	43	25	100	68	48
<i>Entodina nana</i>	17	20	35	28	19	14	38	0	34	24
<i>Dientamoeba fragilis</i>	0	0	4	0	0	0	13	0	2	1
<i>Iodamoeba williamsi</i>	0	11	17	7	0	14	13	0	13	9
<i>Trichomonas hominis</i>	17	14	26	28	19	14	0	50	28	20
<i>Giardia lamblia</i>	17	5	4	7	0	14	25	0	9	6
<i>Chilomastix mesnili</i>	16	8	13	7	6	14	0	0	11	8
<i>Retortamonas intestinalis</i>	0	0	0	3	0	0	13	0	2	1
<i>Palantidium coli</i>	0	3	9	7	6	0	13	0	7	5
Number of patients infected with helminths	67	82	90	72	94	57	100	100	117	82
Number of patients infected with protozoa	33	64	83	63	59	57	50	100	92	65

*Trichuris trichiura* showed the highest rate of infection, an incidence of 78 per cent. This incidence is similar to that of Caldwell, Caldwell and Davis (1930) who found between 80 and 90 per cent infection with trichurids in the "untidy" patients at the Alabama State Hospitals. As will be seen from the table the trichurid infections apparently increased with the duration of hospital residence. This conclusion is further borne out by comparing the incidence in this series with the results obtained by Leathers and Keller (1937) of 0.04 per cent for the state as a whole.

Hookworm infection rate for the state of South Carolina during 1934-1935 was found by Leathers, Keller and Wyman (1936) to be 24.8 per cent. In our series, 66 per cent were infected. This rate and the gradual rise of the incidence during hospital residence indicates that the infection increased during hospitalization.

The *Ascaris lumbricoides* infection was 10 per cent, which compares favorably with the findings of Leathers and Keller (loc. cit.) who found

an incidence of 4 per cent in the state with the rate varying from 0.02 to 11.4 per cent. Apparently, little increase in this infection occurred with hospital residence. In the Alabama mental institutions, Caldwell, Caldwell and Davis (loc. cit.) found that ascariasis did not increase as rapidly as trichuriasis.

The *Strongyloides stercoralis* infections were diagnosed by finding typical rhabditiform larvae in the freshly passed stools. In addition, some of the stools were cultured in bone charcoal; characteristic filariform larvae and adults were recovered from these cultures, both by simple smears and by the use of the Baermann apparatus. Dr. George L. Graham, of the Rockefeller Institute for Medical Research, confirmed the diagnosis of the rhabditiform larvae as *S. stercoralis* and for this courtesy we wish to express our appreciation.

*S. stercoralis* infections were found in 56 of the 142 patients examined, an incidence of 39 per cent. The authors have no information on the incidence of strongyloidiasis in South Carolina but, compared to reports from other localities, the 39 per cent infection found here appears to be high.

No infections of *Enterobius vermicularis* were found. However, as has been shown by several investigators, stool examinations do not reveal the true rate of incidence of this parasite.

*Balantidium coli* is of particular interest among the protozoan infections. This parasite was found in 7 cases of the 142 patients, an incidence of 5 per cent. These infections were reported in detail by Young (1939) and appear to be the first cases reported from South Carolina. As this rarely is reported under normal conditions, it seems that the hospital rate of 5 per cent indicates an increase during residence. All cases occurred in patients of very poor mental state, those who require constant supervision as to habits and general conduct.

#### DISCUSSION

A correlation of the parasitism to the type of psychoses did not reveal anything of significance, especially as the total numbers in the various groups were comparatively small.

The patients examined were housed in one building because of their behavior and general mental state rather than for any specific mental classification, so that the common denominator of the group is that of untidy habits. A description of some of the habits of the patients was given by Young (loc. cit.) in discussing the *Balantidium coli* infections as follows: "Because of their mental condition, all the infected patients tolerated untidiness, both personal and environmental. The clothing might become contaminated and often it was difficult to collect sufficient feces for an examination. This type of patient often puts rocks, leaves,

sticks, grass, dirt and other debris into his mouth. Thus the factors for the transfer of the infections from one person to another were present."

The incidence of parasites in this selected group emphasizes, as has been pointed out by other observers, that when conditions of soil and climate are favorable, any break in personal hygiene completes the cycle of the transmission and leads to a wide dissemination of certain intestinal parasites, especially helminths. It is recognized that such a break in hygiene is found in certain mental patients and as a result, contamination becomes a problem to be constantly guarded against. Many localities in the South have a soil and climate favorable for the development and dissemination of human parasites. In such areas, mental hospitals with their complement of untidy patients, are faced with a task of prime importance in preventive medicine.

#### SUMMARY

A group of patients, who because of mental deterioration were inclined to have untidy habits, were examined for intestinal parasites.

Of 142 examined, 128 (90 per cent) were harboring parasites; 117 (82 per cent) had helminths and 92 (65 per cent) had protozoa. Of these, the most important infections were: *S. stercoralis*, 39 per cent; hookworm, 66 per cent; *T. trichiura*, 78 per cent; *A. lumbricoides*, 10 per cent; and *B. coli*, 5 per cent.

Hookworm, strongyloides, trichuris, and balantidium appeared to increase with residence at the hospital. These findings emphasize again the difficult task in preventive medicine for mental hospitals located in a climate and soil area favorable to the dissemination of intestinal parasites.

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# NATURAL TRANSMISSION OF IMMUNITY AGAINST *TRYPANOSOMA LEWISI* FROM VACCINATED MOTHER RATS TO THEIR YOUNG

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It has been shown by several studies previously reported from this laboratory that mother rats and mice, after recovery from trypanosome infections, transmit to their young specific protective substance. These substances are in all known respects similar to the antibody of the serum, and presumably are identical with it. They are passed to the young largely or entirely through the milk of the mother, and only in small amount, if at all, through the placenta. This last fact is supported by observations that (1) the young of normal animals, if let nurse an immune mother for a day or so, promptly become resistant; and (2) the young of the immune mother, if transferred promptly after birth to a normal mother before ingesting the milk of the immune mother, manifest little or no immunity. The immunity acquired by nursing an immune mother is of comparatively brief duration, being lost by the young wholly or largely within a few weeks after they are weaned (1-4).

The present paper offers the results of a study designed to test whether mother animals immunized not by recovery from infection but by vaccination with a formolized suspension of trypanosomes likewise transmit protective substances to the young in an amount sufficient to protect the young from the specific parasite.

## MATERIALS

*The strain of trypanosome and the preparation of the trypanosome vaccine.*—*Trypanosoma lewisi* was employed in this experiment, the strain being supplied by Dr. L. Reiner of the Burroughs Wellcome and Company, Tuckahoe, New York. The vaccine was prepared in the manner described by Culbertson and Kessler (5): rats 15 days old when infected with *T. lewisi* were bled from the carotid when their infections were near the peak; the citrated blood was slowly centrifugated to separate out its trypanosomes; when freed of erythrocytes, the trypanosomes were suspended in 0.5 per cent formolized physiological sodium chloride solution, and their number determined by count with a hemocytometer. For injection, a suspension containing 4.3 million organisms per cubic centimeter of fluid was employed. The sterility of the vaccine was proved by inoculating young rats with it, with no infection ensuing.

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*The strain of rats.*—The rats were of the Sherman strain, propagated in the Department of Animal Care of this institution. They were fed the same diet, previously described (5), throughout the experiment.

#### EXPERIMENTAL PROCEDURE AND RESULTS

Two normal female rats, Nos. 13 and 21, both 61 days old at the beginning of the experiment, were given a series of 8 injections of *T. lewisi* vaccine at 3-day intervals, 0.1 cc of vaccine being inoculated intraperitoneally per 15 grams of body weight. The two females were put in mating cages with male rats beginning 10 days after the start of vaccination, and left for 10 days. Five days after the last injection of the vaccine, the vaccinated animals, which were then pregnant, were tested for immunity by inoculating living *T. lewisi*. One vaccinated rat proved to be completely resistant, no parasites whatsoever being seen in the tail blood during the next 12 days. The other vaccinated rat showed 2 parasites in 50 microscope fields ( $\times 430$ ) one day after the inoculation of the living forms, but was uniformly negative thereafter when examined daily till the twelfth day (see Table 1 for the results of these tests of immunity,

TABLE 1.—*Demonstrating the immunity against T. lewisi of specifically vaccinated mother rats*

Female rat no.	No. of vaccine injections	Parasite counts on designated days after injecting living <i>T. lewisi</i>						
		1	2	4	6	8	10	12
13	8	0	0	0	0	0	0	0
21	8	+	0	0	0	0	0	0
26	None	+	2+	4+	4+	4+	4+	+
27	None	+	2+	4+	4+	4+	2+	0

\* Two trypanosomes seen in 50 fields of microscope ( $\times 430$ ) on first day only.

Key to parasite counts:

0 = no parasites in 50 microscope fields ( $\times 430$ ).

+ = less than 1 trypanosome per field.

2+ = from 1 to 5 trypanosomes per field.

3+ = from 5 to 50 trypanosomes per field.

4+ = over 50 trypanosomes per field.

along with control animals). On the twelfth day after the immunity test, both females delivered litters, No. 13 producing 7 and No. 21 producing 3 young. Three of the young of No. 13 were—before nursing their own mother—transposed with 4 young of normal mother No. 30, which had delivered 8 young on the same day. The 3 young of immune mother No. 21 were permitted to nurse their own mother. Five days after their birth, all the young were inoculated with 70,000 living *T. lewisi*. The observations upon the ensuing infections are presented in Table 2.

The more significant results were obtained with the young of immune mother 13 and of normal mother 30. All of the own-young and 3 of 4 foster-young nursing immune mother 13 remained consistently negative during the 20 days of observation after the living trypanosomes were

TABLE 2.—*Demonstrating the relative resistance against T. lewisi of young which nurse a vaccinated mother rat compared with that of young nursing a normal mother rat*

Young rat no.	Born of mother no.	Nursed by mother no.	Parasite counts on designated days, after injecting live <i>T. lewisi</i>							
			1	2	4	6	8	11	15	20
1 2 3 4	13 (Immune)	13 (Immune)	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
5 6 7 8	30 (Normal)	30 (Normal)	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			+	0	2+	3+	4+	4+	2+	0
11 12 13	(Immune)	30 (Normal)	0	+	4+	4+	4+	4+	dead	
			0	0	0	+	4+	4+	dead	
			0	0	0	0	+	4+	4+	
			+	+	4+	4+	4+	3+	2+	4+
14 15 16 17	30 (Normal)	30 (Normal)	+	+	4+	4+	4+	4+	3+	2+
			+	+	4+	4+	4+	4+	3+	3+
			+	+	4+	4+	4+	4+	3+	3+
			+	+	4+	4+	4+	4+	3+	2+
21 22 23	21 (Immune)	21 (Immune)	0	0	0	+	0	0	0	0
			0	0	0	0	0	0	0	0
			+	0	0	0	0	0	0	0

Young rats injected when 5 days old with 70,000 *T. lewisi*.  
Key to parasite counts: same as Table 1.

injected. The remaining one foster-young, however, contracted a heavy infection. All of the young which nursed the normal mother, in contrast, contracted heavy infections regardless of whether or not their own mothers were immune. Although there appeared some slight delay in the development of the infection in the 3 foster young nursing the normal mother, their infections finally became quite as severe as those of the own young of the normal mother, two of the foster young succumbing to their infections.

All of the 3 young nursing immune mother No. 21 were found to be highly resistant. Two of these manifested the infection, although in each case on but a single day. The third nursling remained negative throughout the 20 days of observation.

#### DISCUSSION

It is indicated from the results recorded in this paper that mother rats immunized by vaccination with a formolized suspension of *T. lewisi*, transmit to their young the immunity acquired by the vaccination. It is shown that the protective substances responsible for this immunity reach the young largely after birth through the ingestion by the young of the milk of the immune mother, since young born of an immune mother but deprived of her milk by transposition at birth to a normal mother are readily susceptible to severe infections with the homologous parasite.

One detail in the procedure requires further comment. It was pointed

out that five days after the last immunizing injection, the female rats, which were pregnant at that time, were inoculated with living *T. lewisi* to test the production of immunity by vaccination. Although one animal remained completely uninfected, so far as careful tail blood examinations revealed, the other animal showed parasites (2 in 50 fields of the microscope at 430 diameters) for one day following. An objection might be raised, then, that the immunity transmitted to the young resulted from recovery by the mother from an infection with *T. lewisi* rather than from her vaccination with the formolized suspension of this parasite. However, in the opinion of the author, no infection involving the presence of multiplying trypanosomes occurred, the forms found representing merely part of the inoculum of living forms given the preceding day and still in the circulation. Furthermore, it has been shown by Coventry (6) that antibody against *T. lewisi* does not make its appearance in an animal until a significantly heavy infection has been in progress for several days. Parasites were not seen in this work after the first day in the one mother animal which showed them at all. It is concluded, therefore, that the immunity transmitted by these mother rats was developed in them by vaccination and not by recovery from infection.

The character of the antibody transmitted from the mothers to the young probably depends on the character of the antibody in the blood of the mother at parturition and during the nursing period. Taliaferro (7) has shown that rats which have recovered from infection with *T. lewisi* produce two distinct serum antibodies—the one manifesting a trypanocidal action and the other a capacity for inhibiting the reproduction of trypanosomes. He has also demonstrated that the reproduction-inhibiting antibody is developed principally although possibly not exclusively by rats through vaccination. Some small amount of evidence is forthcoming from the present work to indicate that the antibody transmitted to the nurslings from the vaccinated mother rats inhibited the reproduction of the trypanosomes in the nurslings. Of the three young nursing Mother Rat No. 21, for example, two revealed trypanosomes in the blood, one of these (young rat No. 21) as many as six days after the inoculation of living forms. Evidently, the trypanosomes, although present during this six-day period in this animal were not destroyed by the substances obtained through nursing the immune mother, but were merely prevented from increasing in number. Whether the trypanocidal antibody also was transmitted to some of the other nurslings and was responsible for the complete absence of trypanosomes from their blood was not determined.

#### SUMMARY

Mother rats immunized against *Trypanosoma lewisi* by vaccination with a formolized suspension of this organism transmit their immunity to

their young. The young become immune largely by ingesting the milk of the immune mother. Such immunity as the young may acquire through the placental circulation is largely lost within a few days after birth.

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## THE FUNCTION OF RESPIRATORY PIGMENTS OF CERTAIN TURTLE PARASITES\*

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Von Brand (1938) discusses in some detail most of the previous work in the field of the respiration of parasitic worms, in an attempt to answer the question whether helminths in the intestines of vertebrates lead an aerobic or anaerobic life. Because of the low oxygen tensions observed in the vertebrate alimentary canal (Tappeiner 1883, Long and Fenger 1917, von Brand and Weise 1932), the worms were considered to have anaerobic metabolism. Anaerobic respiration is known to occur in all of the parasitic worms which have been tested (Wardle 1937a, von Brand 1937b, Stannard, McCoy and Latchford 1938, and many earlier works). Likewise aerobic respiration has been shown to exist. Von Brand (1938) concludes that, "large helminths, like *Ascaris* or *Moniezia*, gain their energy predominantly by fermentation [of glycogen] during their life in the intestine, but that small parasites like sheep nematodes, may lead there a more aerobic life."

Several investigators (Davey 1938, Slater 1928) show that worms in vitro live longer under aerobic than anaerobic conditions. Hoeppli, Feng, and Chu (1938) give a complete reference list of studies of this type. Von Brand (1937b) has shown that *Ascaris* has the ability of re-synthesizing glycogen from the breakdown products of anaerobic metabolism when plenty of oxygen is available.

Both hemoglobin and cytochrome have been discovered in nematodes (Keilin 1925), but only cytochrome has been found in cestodes (Friedheim and Baer 1933). Hemoglobin was found to be concentrated in the hypodermis of *Ascaris* (Krüger 1936) where it allegedly aids the organism by removing oxygen from the surrounding medium through the cuticle. *Trichina* larvae were shown to have hemoglobin (Stannard, McCoy, and Latchford 1938) which probably aids somehow in their respiration. Von Brand (1937a) found hemoglobin in another larval nematode.

Aducco (1889) carried out several experiments on the red pigment in the cuticle and body fluids of *Diectophyme renale* (Goeze 1782). The pigment was found to be similar to vertebrate hemoglobin, but much more resistant to change by physical and chemical agents. The pigment from *D. renale* could not be completely reduced with a vacuum pump, but

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with the aid of chemical reducing agents, complete reduction of it was accomplished.

Wardle (1937a and b), von Brand (1933, 1937b, 1938), and others have shown that the oxidizable substrate in parasites is glycogen and that when energy is produced, the glycogen is fermented to  $\text{CO}_2$  and lower fatty acids. Krüger (1936) has shown the acids to be valeric and capronic in the case of *Ascaris*. In the presence of oxygen, aerobic oxidation also takes place.

Very little work has been done on the oxygen-obtaining mechanisms of parasites. Wells (1931) has demonstrated that a hookworm sucks blood from the host which would permit it to obtain oxygen from erythrocytes. Von Brand (1938) points out that 0.2 mg of  $\text{O}_2$  would be available daily to each worm from this source.

As has already been mentioned, respiratory pigments are known to occur in parasites, but the mechanics of their action have not been determined, hence an attempt to discover the role of hemoglobin in the respiration of turtle parasites was undertaken.

#### MATERIALS AND METHODS

Two trematodes (*Telorchis robustus*, *Allasostoma magnum*) and three nematodes (*Camallanus trispinosus*, *Falcaustra affine*, and *Cruzia testudinis*) were tested for hemoglobin. A suitable number of worms, equivalent to about 1 cc in volume, if that many were available, were removed from the host, washed from 1 hour to 48 hours in salt solution at 4° C, placed in a 15 cc centrifuge tube and frozen in brine. Then 0.5 cc of phosphate buffer (pH 6.8) and 0.5 cc of distilled water were added to the tube, and the contents repeatedly frozen and thawed, usually five or six times, until there was no color remaining in the worms. The solutions were centrifuged and the clear supernatant fluid was removed by a pipette to a test tube. If the worms contained hemoglobin, then this was the hemoglobin solution used in the experiments. The residue from some extractions of *C. trispinosus* and *A. magnum* were re-extracted with 1.5 cc of weak ammonia solution containing a small amount of sodium hydro-sulfite. This was centrifuged and the supernatant fluid was tested for cytochrome.

Spectroscopic examinations were conducted in three ways: (1) the solution was spectrographed and the plate analyzed by a photoelectric densitometer; an iron arc spectrum was photographed on the same plate for reference (Dr. L. G. Bonner assisted in this phase of the work); (2) the solutions were examined by eye with a comparometer spectroscope; (3) a Zeiss microspectroscopic eye piece was used with an ordinary compound microscope to detect hemoglobin in individual worms.

An oxygen dissociation curve of a single sample of the hemoglobin

from *C. trispinosus* was determined with the aid of Dr. F. G. Hall and his micro-method for dilute hemoglobin solutions (Hall 1934, 1935).

The ability of *C. trispinosus* to reduce its own hemoglobin was determined in two ways: (1) several worms were removed from a turtle, washed in salt solution, placed in a fine capillary tube which was filled with saline and closed with paraffin at both ends. The worm was examined with a microspectroscope. Later the tube was broken, the worms removed and examined in the same manner in a small drop of saline which was exposed to the atmosphere; (2) a spectro-comparator such as described by Hall (1935) was used in place of the ocular of a compound microscope. On the mechanical stage of the microscope was fastened a fine glass tube containing a worm for examination, which was connected with a reservoir of saline so that fluid could be passed through the tube at any desired speed; the spectral bands of the worm could thus be matched by absorption bands of oxi- and reduced-hemoglobin solutions of known proportions.

#### RESULTS

Reddish extractions were obtained from *Telorchis robustus*, *Allasostoma magnum*, and *Camallanus trispinosus*. When these solutions were examined spectroscopically, they were found to have two absorption bands, one at about 5,750 Ångström units and the other at about 5,400. These bands disappeared when the solutions were reduced with sodium hydrosulfite, and were replaced by a single broad band in a region between the two former bands (Figs. 1 and 2). Hemoglobin solutions

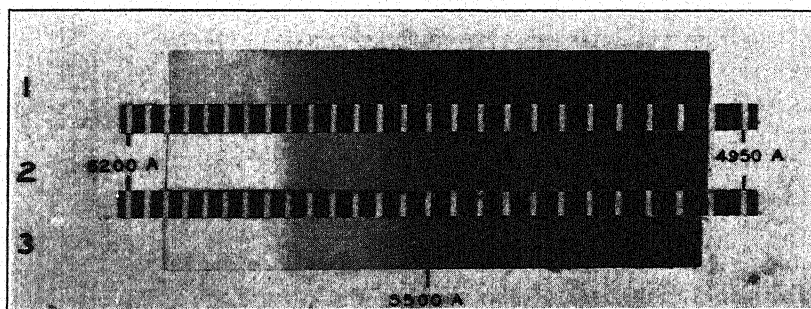


FIG. 1. Spectrographs of hemoglobin from *Telorchis robustus*. 1. Reduced hemoglobin. 2. Partially reduced hemoglobin. 3. Oxy-hemoglobin.

behave in this manner, hence it is assumed that hemoglobin lends the reddish color to these solutions. *Falcaustra affine* and *Crusia testudinis* were not found to have hemoglobin.

The second extracts from *A. magnum* and *C. trispinosus* showed when reduced a faint absorption band at 5,500 Ångström units which was probably the strong band of cytochrome C.

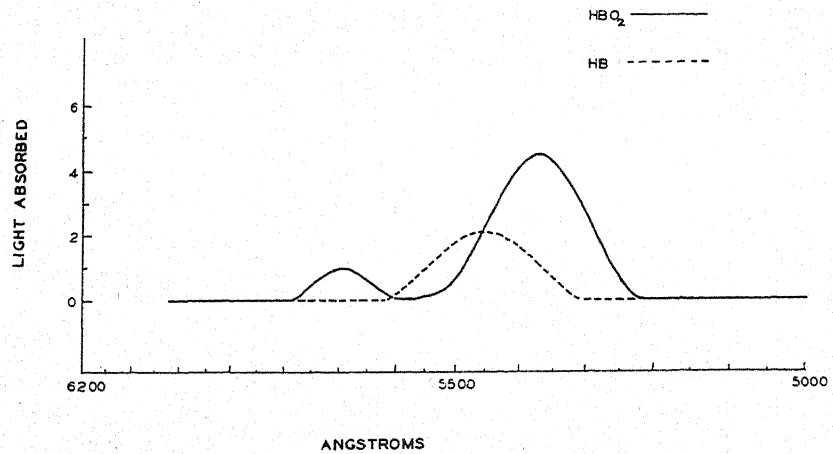


FIG. 2. The relative shape and position of the absorption bands of *Telorchis robustus* hemoglobin. Amount of light is given in arbitrary units. The position of the bands is not accurate to more than 100 Ångström units.

The oxygen dissociation curve obtained for the hemoglobin of *C. trispinosus* is given in Fig. 3, along with the curve of the host hemoglobin as determined under similar conditions by Wilson (1938). The curve for the parasite pigment is far to the left of that of the host.

When in anaerobic surroundings, the hemoglobin of *C. trispinosus* is in a reduced state, but when the worm is in contact with oxygen, the hemoglobin is in a partially oxidized condition, the extent of oxidation depending upon the partial pressure of the oxygen.

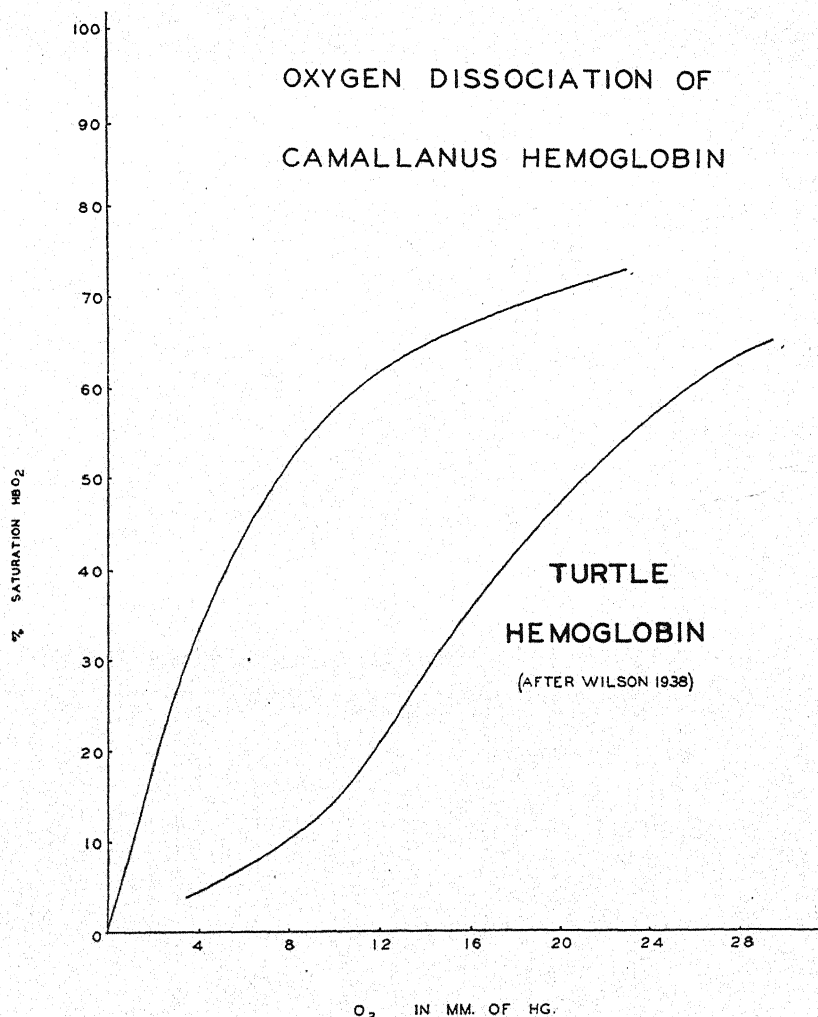
*T. robustus*, *A. magnum*, and *C. trispinosus* cling to the intestinal wall by their mouths, where they cause inflammation. When these worms are removed from their points of attachment, their former positions are at times indicated by small red "pimples." *F. affine* and *C. testudinis* live free in the lumen of the gut.

#### DISCUSSION

That a parasite has hemoglobin not identical with that of its host is shown by the difference in the two oxygen dissociation curves (Fig. 3). Whether or not the raw materials for the manufacture of hemoglobin by the parasite are obtained by digesting host hemoglobin and absorbing the decomposition products, is not known, nor is it important in the consideration of the oxygen-obtaining mechanism. Telorchids usually live just posterior to the bile ducts, so perhaps they obtain their essential substances for synthesis from the bile.

Turtle parasites that contain hemoglobin are all in close contact with the blood stream of the host. The dissociation curve of the host pigment is far to the right of that of at least one of the worms, which signifies that





\* FIG. 3. Oxygen dissociation curves. Hemoglobin from *Camallanus trispinosus* and its host *Pseudemys troostii* tested under similar conditions.

the hemoglobin of the parasite can take up oxygen at very low pressures or even from the venous or reduced blood of its host. The work of Aducco (1889) on the red pigment of *D. renale* showed that its affinity for oxygen was much greater than that of vertebrate hemoglobin. In fact the properties of the red pigment of *D. renale* agree so closely with those of the worm hemoglobins discussed in this paper, that there is little doubt that the pigment described by Aducco is in reality hemoglobin. *C. trispinosus* can reduce its own hemoglobin, probably with the aid of cytochrome which acts as an oxygen transport mechanism between the



hemoglobin and the oxidizable substrate (glycogen ?, von Brand 1938). These facts show that it is possible for oxygen to be taken from the host blood by the hemoglobin of *C. trispinosus*, and that this stolen oxygen can be used in the metabolic processes of the worm. That this occurs is supported by the fact that those worms which live free in the lumen have not been found to contain hemoglobin. It can not be said that this mechanism has actually been demonstrated for *C. trispinosus*. It has been shown to be physically possible even probable, but until some means of observing the phenomenon in its entirety is devised, final judgment must be withheld. A mammalian embryo has a similar mechanism for obtaining its oxygen from its mother's blood (Evans 1936).

#### CONCLUSIONS

1. Hemoglobin is present in *Telorchis robustus*, *Allasostoma magnum* and *Camallanus trispinosus*.
2. Cytochrome is present in *A. magnum* and *C. trispinosus*.
3. Hemoglobin is probably not present in *Falcaustra affine* or *Crusia testudinis*.
4. Host and parasite hemoglobin are not identical.
5. The respiratory pigments of these parasites probably aid them in obtaining oxygen from the host blood.

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## RESEARCH NOTES

### INCIDENCE OF *ENDAMOEBA HISTOLYTICA* AND INTESTINAL NEMATODES IN A GEORGIA STATE INSTITUTION

Through the kindness of Dr. John W. Oden, Superintendent of the State Hospital at Milledgeville, Georgia, a parasite survey was made during 1938 on a number of patients at the institution. One ward of 72 white adult females was examined in June for intestinal parasites, and again in November when the ward contained 70 patients. Mentally the patients examined fell into various classifications; physically all were considered to be healthy adults and had been in residence at the institution from several months to many years. An additional ward of 88 white females was examined in October for pinworm infection only. No information concerning the mental or physical status or length of institutional residence of these patients was obtained.

The parasite examinations were made as follows: For *Endamoeba histolytica* and *Strongyloides stercoralis* saline smear and iodine smear examinations from 3 stools per patient; for worms except *Strongyloides stercoralis* and *Enterobius vermicularis* brine flotation examinations from 2 stools per patient; for *Enterobius vermicularis* 3 anal swabs per patient.

Concurrent with the parasite examinations Stoll egg counts on 3 stools per patient were made to determine the intensity of certain of the worm infections. These determinations were calculated from the data of Leathers and Keller (1935, New Orleans Med. and Surg. J. 87: 425-435) and Keller and Leathers (1936, Am. J. Hyg. 23: 216-230).

The results of the parasite examinations are summarized in the following table. No tapeworm infections were encountered.

TABLE 1.—Record of positive diagnoses for intestinal parasites

Date	Patients examined	<i>Endamoeba histolytica</i>	<i>Ascaris lumbricoides</i>	<i>Necator americanus</i>	<i>Trichuris trichiura</i>	<i>Strongyloides stercoralis</i>	<i>Enterobius vermicularis</i>
1938	No.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
June . . . . .	72	40	14	33	65	6	53
November . .	70	44	9	30	61	6	69
October . . .	88	*	*	*	*	*	27

\* No data obtained on these parasites.

The parasite findings given above have been analyzed as follows:

*Endamoeba histolytica*. The importance of the high incidence of this parasite appears to be minimized by the fact that there was no diarrhea or dysentery among the patients examined and for the most part all stools observed were normal, formed stools. In patients of the type examined only such objective symptoms as diarrhea or dysentery could be used as an index of the pathogenicity of the parasite and the complete absence of such symptoms suggests that the organism was one of low virulence or that a resistance had been acquired due to long-continued exposure implicit in the insanitary habits of this type of patient.

The analysis of the nematode findings was based on the egg counts made during the June examinations. A similar analysis made in November revealed no appreciable differences and the data are therefore not cited.

*Ascaris lumbricoides*. The egg counts of the 10 positives for this parasite indicated that all infections were light.

*Necator americanus*. In only one case did the egg counts for this parasite indicate a heavy infection. Two cases were considered moderate infections and the remainder light.

*Trichuris trichiura*. With this parasite 5 cases out of 47 were regarded as heavy or very heavy infections. Seventeen were considered moderate and the remainder light.

No estimation of intensity of infection with *Strongyloides stercoralis* was possible. Nor could intensity of infection with *Enterobius vermicularis* be gauged since the number of ova on a swab is no index of the number of worms in the intestine.

The foregoing analysis of the findings for both *E. histolytica* and for the helminth parasites indicate that the parasite burden of the patients was, on the whole, light although the incidence of parasitism was high. In an institution of this type a high incidence of intestinal parasites is not necessarily surprising in view of the fact that the communal domiciling of mentally deranged persons provides very favorable conditions for the spread of parasitic infections.—LUCY V. REARDON, *Associate Protozoologist, National Institute of Health, U. S. Public Health Service, Washington, D. C.*

#### INTESTINAL PROTOZOA IN 106 PARASITOLOGY STUDENTS

In a survey of a class of 45 parasitology students at Dartmouth College, Connell and French (1939, J. Am. Med. Assn. 113: 649-652) reported 3 with *Entamoeba histolytica* and 10 with one or more species of amoeba.

Similarly, as a regular feature of the work in the course in protozoan parasitology at the University of Pennsylvania, each student is given a fecal examination for intestinal protozoa. In Table 1 are summarized the results of several years' efforts. One normally passed stool was obtained from each student and the specimens averaged 17.1 hours old at the time of examination. The diagnoses were made from saline, iodine and permanent preparations of each sample.

TABLE 1.—Percentage of protozoa in 106 students

Positive for protozoa	Negative for protozoa	<i>Entamoeba histolytica</i>	<i>Entamoeba coli</i>	<i>Endolimax nana</i>	<i>Dientamoeba fragilis</i>	<i>Giardia lamblia</i>	Multiple infections	<i>Blastocystis hominis</i>
34	66	8	8	13	3	11	8	69

The absence of such forms as *Iodamoeba*, *Trichomonas*, etc is probably due to the relatively small number examined. Of these 106 students, 59 were residents of Philadelphia, while Pennsylvania, New Jersey and Delaware accounted for 94. Of the 8 harboring *E. histolytica*, 5 were Philadelphians.—ROBERT M. STABLER, *Department of Zoölogy, University of Pennsylvania*.

#### THE OCCURRENCE OF LARVAE OF THE STABLE FLY, *MUSCINA STABULANS* (ZETT.) IN LIVING NYMPHS OF THE GRASS-HOPPER, *XANTHIPPIUS CORALLIPES PANTHERINUS* (SC.)

During the course of some studies of the immature stages of certain grasshoppers, two second-instar nymphs of *Xanthippus corallipes pantherinus* (Sc.) were observed in the field near Dallas, Texas, on November 9, 1939 to be unusually inactive and upon immediate dissection of these living specimens five larvae of *Muscina stabulans* (Zett.) were obtained from each nymph. Five other nymphs collected at the same time were confined in screen-tight laboratory cages and upon dissection five days later, three were similarly infected.

Collections made in the same locality on April 20, 1940 revealed that three of four fourth-instar nymphs and five of seven fifth-instar nymphs were infected. Likewise three days later similar collections showed two of seven fourth-instar nymphs and six of eight fifth-instar nymphs to be infected. In all instances the infected individuals exhibited the same retarded activity. Collections of seven adults on May 12 and eight adults on May 21 failed to reveal any larvae.



Mr. D. G. Hall has kindly identified the species, from adults reared from the larvae, as *Muscina stabulans* (Zett.).

When dissected out, the larvae are active and inclined to crawl several inches or feet, whereupon the older individuals pupate, although in the laboratory cages three actually penetrated weakened living nymphs of two other grasshoppers, *Encyptolophus sordidus costalis* (Sc.) and *Chortophaga viridifasciata* (DeGeer). The number of maggots in a nymph varied from one to eighteen, with a mean of eight.

In laboratory cages pupation occurred (90 specimens) from April 29 to May 6. The pupae were usually embedded in the soil from one-half to one and one-half inches, although nine were found on the surface of the soil under dead vegetation. The adults emerged from May 7 to May 12.

Many reports of the rearing of this fly from various orders of insects have been recorded in the literature, although its actual status as a parasite is often disputed, since no records are to be found which record a complete parasitic part of the life cycle. The fact that the larvae can be found in living, moving nymphs, however, would seem to give additional support to the view that the fly is, under these conditions, actually parasitic, possibly entering the host as a larva, and may represent a transitional stage in the evolution of parasitism.—HERBERT KNUTSON, *Biology Department, Southern Methodist University, Dallas, Texas.*

#### TAMERLANEA BRAGAI, A PARASITE OF PIGEONS IN PUERTO RICO

During October, 1938, one of us (J. M.) recovered a trematode from a pigeon which came from a farm at Adjuntas, central Puerto Rico. Nineteen of twenty birds in this locality harbored the parasite. Considerable difficulty was at first experienced in the identification of the fluke. An examination of the figure of *Tamerlanea bragai* Santos (Reis and Nobrega, 1936, Doenças das Aves, Sao Paulo, Fig. 228, p. 313) convinced us we were dealing with this species, an opinion confirmed by Dr. E. W. Price and Mr. A. McIntosh of the U. S. Bureau of Animal Industry. Specimens kindly forwarded by Professor Santos from Brazil were received in poor condition and could not be satisfactorily stained to permit comparison.

The parasite was restricted to the kidney and urethra. The number of flukes recovered varied from two to twenty. Pigeons kept in cages for several months appeared unaffected by their infections. An inflamed urethra was noted in one pigeon at autopsy. According to Dr. Krakower of the School of Tropical Medicine: "The effects of the parasite upon the kidneys are chiefly mechanical, that is, by dilation of the pelvis and collecting tubules, without, however, interfering with the renal function. The dilation is sufficient to allow this. The muscular hypertrophy of the walls is compensatory, probably to expel the parasite from the lumen. The slight excess of round cell infiltration indicates that the irritation set up by the parasite is minimal."

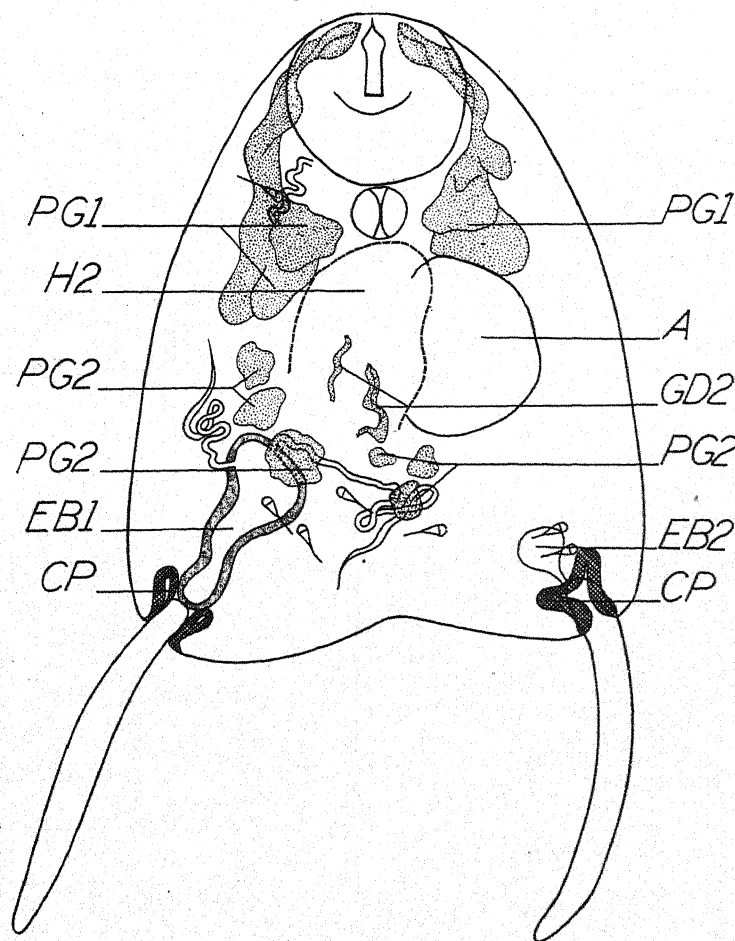
Though first found in the interior of the Island, the recovery of adult flukes from autopsied pigeons at Rio Piedras, and of the typical small brown ova from the excreta of the pigeons in several localities between San Juan and Rio Piedras, suggest that the species is widely distributed in Puerto Rico. This marks the second time a trematode known from South America has been found in Puerto Rico without the interpolation of any known intermediate distributional records. The first instance was that of *Platynosoma concinnum*, common locally in the livers of cats.

The ova of *T. bragai*, when passed, contain fully developed miracidia which fail to hatch in water or charcoal cultures. This seems to indicate ingestion by the molluscan host.—JOSÉ F. MALDONADO, *Insular Agricultural Experiment Station, Rio Piedras, P.R.* AND W. A. HOFFMAN, *Department of Medical Zoology, School of Tropical Medicine, San Juan, P.R.*

## PARTIAL TWINNING IN A STYLET CERCARIA

In the course of a study of development of the cercaria of *Alloglossidium corti* an abnormal nearly mature cercaria was encountered in material obtained by dissection of the sporocyst.

As shown in the figure (composite drawing from both living and stained speci-

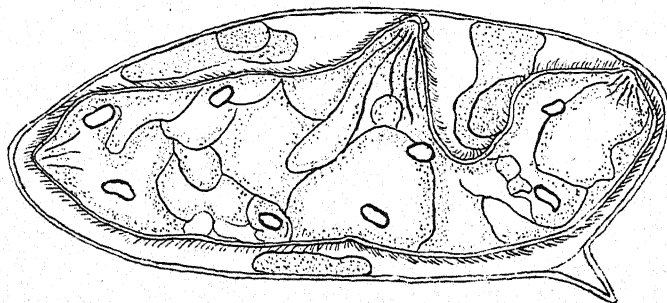


men), the posterior part of the cercaria is almost completely doubled. Both tails were actively moving and appeared normal, although the movement of the cercaria as a whole was rather sluggish when compared with normal cercariae of a similar stage of development. Caudal pockets (cp) are distinct around both tails. There is a partially developed second head region (h 2) near the acetabulum (a). The apparently normal head region has stylet, oral sucker, pharynx and ducts of penetration glands; the penetration glands (pg 1) have indistinct boundaries; intestinal caeca are not visible. Penetration glands (pg 2) and their ducts (gd 2) associated with the second head region (h 2) were observed. A second stylet was noted by one observer, but was not seen again in the living specimen, or after staining. Of the two excretory bladders one (eb 1), large and thick-walled, appears normal and receives two collecting tubes; the other (eb 2) is small, with indistinct wall and no visible collecting tubes. Several flame cells were visible but the flame cell pattern could not be determined.

It is entirely possible that such an abnormal cercaria would never emerge from the snail.—KATHLEEN L. HUSSEY, *Department of Zoology, University of Michigan.*

#### MIRACIDIAL TWINNING IN *SCHISTOSOMA MANSONI*

While making examinations of the excreta of a patient infected with *Schistosoma mansoni*, an egg of normal size was found containing a pair of miracidia (See Fig.). The miracidia were fused anteriorly for about one third of their



length and in each of them a normal set of four active flame cells was observed. The head was located in the middle portion of the egg, the posterior end of the miracidia situated at each extremity. The paired organisms were quite active.

Miracidial twinning has been reported in excreta from a monkey (Hoffman and Janer, 1936, *Proc. Helm. Soc. Wash.* 3: 62). At the time it was believed that the host may have in some way caused this abnormality. The present finding indicates the phenomenon may occur in man, as well.

Penetration of the molluscan host by these miracidia, were they more abundant, might give rise to some interesting genetic conditions.—JOSÉ L. JANER, *Department of Medical Zoology, School of Tropical Medicine, San Juan, P.R.*

#### A STOMACH TUBE FOR MICE

Male catheters are in general use as stomach tubes for rats, guinea pigs, and rabbits. These tubes, however, are not of sufficiently small diameter to be used for mice. Glass tubes have proved unsatisfactory in the hands of the author due to lack of flexibility of the glass. A small stomach tube has therefore been improvised from cellophane and so far has proved successful in use on mice.

Different grades of cellophane are available commercially, but the author has used only one type, the cellophane ordinarily used for wrapping purposes. This is about the same weight as that used on cigarette packages. The cellophane is cut into trapezoidal strips (Fig. 3) and, after one side of the lower right hand corner has been dampened, the edge is rolled (Fig. 2) between the thumb and index finger. The angle at which the cellophane is rolled (Fig. 2, 115°) in relation to the longitudinal axis of the paper will determine the length of the resulting tube and consequently the number of layers composing the wall. After completion of the rolling process, the distal end of the paper is cemented and since the tube is rolled tightly, this is sufficient to hold it together and keep it from telescoping.

From the experience of the author, a very useful tube is made by starting at an angle of approximately 115° on a cellophane strip of the size shown in Fig. 3. After the cellophane has been rolled to form a tube approximately  $\frac{3}{4}$  inches in length, the angle of rolling is increased slightly each time the tube is turned so that a tube results (Fig. 1) which measures about  $3\frac{1}{4}$  inches long. One quarter inch is then cut from the small end and  $\frac{1}{2}$  inch from the large end. The resulting tube has a diameter at the larger end of approximately 2 mm and a diameter at the smaller end of slightly less than 1 mm. The edge of the small end should be smoothed with fine sand paper. A hypodermic needle of the proper size, with the point filed off, is inserted into the large end of the tube when in operation.

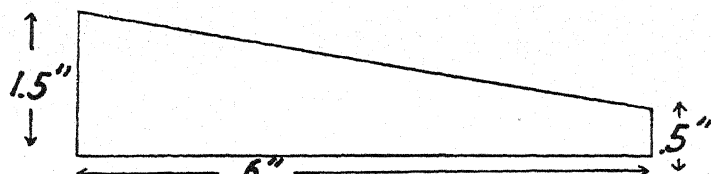


FIG. 3

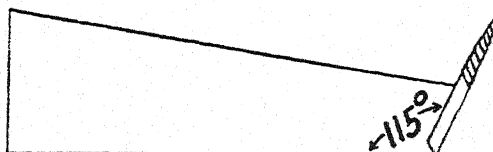


FIG. 2

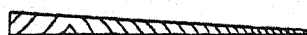


FIG. 1

Mice are difficult to restrain manually so a restraining bag with a zipper, stocks, or some other device should be used for holding the animal. It is necessary to press on the extreme base of the mandible on either side in order to keep the mouse's jaws apart and hence avoid having the tube bitten through. This pressure is most easily applied by the fingernail of the index finger while the thumb holds the back of the animal's head. Care should be taken to avoid pinching the trachea. Some practice is necessary in order to apply pressure correctly. In an alternative method a small glass tube is inserted into the mouth and provides a passageway for the stomach tube thus protecting it from the incisors.

The stomach tube, previously coated with a lubricant, should be inserted by following mid-dorsally down the roof of the mouth, exercising care to avoid contact with the molars and tracheal orifice. The insertion of the needle into the stomach tube and injection should be accomplished as quickly as possible since mice seem unable to breathe while the stomach tube is in situ.

The author is using this tube for the administration of anthelmintics to mice but it should serve equally well for infectious material, vitamin concentrates, etc.—  
G. M. SPURLOCK, *Division of Entomology and Parasitology, University of California, Davis, California.*



## REVIEW

THE INVERTEBRATES: Protozoa through Ctenophora. By Libbie Henrietta Hyman. xii + 726 pp. 221 illustrations. McGraw-Hill Book Company, Inc., New York. 1940. \$7.00.

This book is part 1 of volume I of a projected three-volume reference work on the invertebrates. It opens with two introductory chapters, the first (21 pp) dealing with protoplasm, the cell and the organism; and the second (18 pp) with classification. Chapters III to VIII deal respectively with Phylum Protozoa (90 pp); Phylum Mesozoa (15 pp); Introduction to the Lower Metazoa (35 pp); Phylum Porifera (80 pp); Phylum Cnidaria (296 pp) and Phylum Ctenophora (35 pp). Each chapter includes at its end a fairly extensive bibliography and the volume closes with an index of 30 pages.

Chapter I begins with a consideration of the physical, chemical and biological properties of protoplasm and continues with a discussion of the cell and its parts, the cell theory and cell division. The organism is then considered with attention to form, symmetry, metamerism, polarity, etc.

In Chapter II, on classification, the subject is treated historically and then a system is adopted which recognizes 22 phyla. Subkingdom I consists of Phylum Protozoa. Subkingdom II, the Metazoa, comprises three branches: A, Mesozoa (Phylum Mesozoa); B, Parazoa (Phylum Porifera); and C, Eumetazoa. The latter consists of Grade I, Radiata, composed of the phyla Cnidaria and Ctenophora, and Grade II, Bilateria. This grade consists of three groups: a, Acoelomata (Phyla Platyhelminthes and Rhynchocoela or Nemertinea); b, Pseudocoelomata (Phyla Aschelminthes—rotifers, nematodes, etc.—and Entoprocta); and c, Eucoelomata. The Eucoelomata are further divided into 1) The Schizocoela, in which are included the Phyla Bryozoa or Polyzoa, Phoronida, Brachyopoda, Mollusca, Sipunculoidea, Priapulidae, Echiuroidea, Annelida and Arthropoda; and 2) The Enterocoela which contains the Phyla Chaetognatha, Echinodermata, Hemichorda and Chordata.

Chapter III on Protozoa begins with a section on the characters of the phylum in which it is stated that they are acellular rather than unicellular, followed by an outline of classification, and a discussion of the general morphology and physiology of the group. This valuable discussion (34 pp) includes the topics of form and size, general structure of the body, the nucleus, encasements and shells, kinetic elements, locomotor organelles, nutrition, respiration and the contractile vacuole, neuromotor system and sense perception, behavior, encystment, nuclear phenomena of division, asexual reproduction and the division of the cytosome, sexual reproduction, regeneration, genetics, and ecology. Under ecology the terms symbiosis, commensalism and parasitism are defined and discussed.

In the classification of the Protozoa the phylum is first divided into the Subphyla Plasmodroma and Ciliophora. The Plasmodroma include the Classes Flagellata or Mastigophora, the Rhizopoda or Sarcodina, and the Sporozoa. The Flagellata consist of the Orders Chrysomonadina, Cryptomonadina, Dinoflagellata, Chloromonadina, Euglenoidina, Phytomonadina or Volvocales, Protomonadina, Polymastigina, Hypermastigina and Rhizomastigina or Pantostomatida. The Class Rhizopoda includes the Orders Amoebozoa or Lobosa, Foraminifera, Heliozoa and Radiolaria. The Mycetozoa are relegated to the Fungi and hence omitted. The shelled Protozoa are placed in the Foraminifera. The Sporozoa are divided into four Subclasses: 1) The Telosporidia, consisting of the Orders Gregarinida, Coccidia and Haemosporidia; 2) The Cnidosporidia, consisting of the Orders Myxosporidia, Actinomyxidina and Microsporidia; 3) Sarcosporidia, which includes the Orders Sarcosporidia and Globidia; and 4) Haplosporidia, which is not divided into orders.

In the Subphylum Ciliophora, two Classes, Ciliata and Suctoria are recognized. The Ciliata are divided into the Subclasses Protociliata and the Euciliata. The latter is divided into four Orders. The Order Holotricha contains the Suborders Gymnostomata, Trichostomata, Hymenostomata, Astomata and Apostomea. The Order



Spirotricha includes the Suborders Heterotricha, Oligotricha and Hypotricha. The Peritricha and Chonotricha are recognized as separate orders.

Each major taxonomic section opens with a definition of the group, a statement of its general characters and a discussion of its morphology and physiology; then follows an account of the morphology, life history, etc of members of the successive orders. The chapter closes with a section on general and phylogenetic considerations and a bibliography of nearly 25 pages set solid.

As with the other chapters, the one on Protozoa is extensively illustrated. There are 60 figures in this chapter but most of these are full-page groups of drawings so that a total of several hundred drawings is represented. Most of the drawings have been redrawn from a variety of sources but many are original having been drawn from actual specimens.

Typographical errors have been reduced to a minimum, but there are occasional statements with which one might disagree. For example, on page 13 it is stated that the mitotic spindle is seen only in fixed preparations: "The spindle and astral fibers apparently do not exist as such in life but may be fluid channels in the cytoplasm." There are a good many statements to the contrary in the literature and one might refer to the work of Cleveland and his associates who state that the spindle fibers can be plainly seen in living specimens of *Barbulanympha*. On page 25 it is stated that "Das Thierreich" was begun in 1896, whereas one section of this work appeared as early as 1873. However, considering the amount of ground covered, the information is remarkably well-digested and up-to-date.

As is to be expected in a general reference work such as this, the parasites are not given disproportionate attention, although by no means neglected. Those groups consisting entirely of parasites such as the Hypermastigina, Astomata and Sporozoa are presented with as much detail as other groups. In sections containing few or many parasites, these are given attention proportionate to their numbers and importance. For example, in the section devoted to the Order Protomonadina, the discussion of the haemoflagellates, because of their importance, makes up the bulk of the section.

Only one other group covered by this volume is of special interest to parasitologists and that is the Phylum Mesozoa. To the reviewer the chapter on this enigmatic group seems adequate and modern.—D. H. WENRICH.

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No. 4, August 8

No. 5, October 9

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## VARIATION IN A NEW SPECIES OF CESTODE, *RAILLIETINA (SKRJABINIA) VARIABILA*, FROM THE PRAIRIE CHICKEN IN ILLINOIS

W. HENRY LEIGH\*

In the course of studies carried on by the Game Management Section of the Illinois Natural History Survey relative to the status of the prairie chicken in central Illinois, fourteen young and fourteen adult birds were collected for studies of parasites and diseases (Leigh, 1940).

Ten of the fourteen young prairie chickens collected during two summers were infected with cestodes of the genus *Raillietina*. Four of the infections were so intense as to occlude the lumen of the small intestine for one-half to two-thirds its length. What effect such intense infection has upon the young hosts is not known at present. It seems reasonable that the minimum effect would be a reduction in reserve strength and vitality that might make the young more susceptible to predation or secondary infections. Finding no cestodes in the adult birds seems to indicate that the prairie chicken is susceptible to this species of *Raillietina* only or largely during the first few weeks of life.

Attempts to identify these cestodes led to the conclusion that they had not been described previously. Due to the contrast in general appearance and the variation of many morphological characters in the cestodes of several hosts, it was at first thought that the collection comprised several species. However, detailed studies revealed intergrading individuals between the extreme variants that made it clear that only one mutable species was present. This, the first representative of the genus to be recorded from the prairie chicken, is described in this paper under the name *Raillietina (Skrjabinia) variabila*.

### PROCEDURE

Measurements comprising Table 1 and those used in formulating the specific diagnosis were made on only those cestodes which remained

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\* Thanks are due Dr. R. E. Yeatter of the Illinois Natural History Survey for excellent cooperation in all phases of the problem, and to Prof. H. J. Van Cleave of the University of Illinois for critical evaluation of the results of the study.

intact, possessed gravid proglottids and were in an excellent state of preservation, namely those cestodes from hosts numbered 1, 7, and 8. The remaining part of the collection was carefully studied, but since the strobilae were immature or broken up, the data were not used in preparing the specific diagnosis.

TABLE 1.—*Synoptic characters of three differing groups of cestodes from three different hosts\**

	Group 1	Group 2	Group 3
Total length, mm . . . .	6 (47-84) 71	5 (129-186) 147	4 (220-272) 247
Greatest width, mm . .	11 (0.6-2.1) 1.3	5 (1.3-1.6) 1.5	4 (2.6-2.8) 2.7
Size gravid proglottids, mm . . . . .	5 (0.5-1.4 by 0.3-1.1) 0.9 by 0.5	4 (1.4-2.0 by 0.5-0.7) 1.9 by 0.6	4 (1.8-2.2 by 1.3-1.6) 2.1 by 1.5
Number rostellar hooks	3 (240-260) 252	3 Approx. 250	3 (256-272) 265
Size rostellar hooks, $\mu$ . .	1st row: 17-19 2nd row: 15-16	1st row: 18 2nd row: 15	1st row: 18 2nd row: 15-16
Size cirrus pouch, $\mu$ . . .	9: 25 (84-117 by 31-51) 90 by 39	5: 20 (108-117 by 36-51) 113 by 45	4: 20 (141-171 by 57-72) 159 by 64
Number testes . . . . .	12: 75 (33-65) 43	4: 30 (56-77) 65	4: 30 (50-82) 68
Size testes, $\mu$ . . . . .	7: 35 (24-39) 32	5: 30 (39-60) 50	4: 30 (69-102) 82
Size capsule, $\mu$ . . . . .	2: 10 (60-84 by 42-57) 72 by 47	1: 12 (69-78 by 45-72) 72 by 61	1: 17 (63-102 by 45-75) 86 by 59
Size embryo, $\mu$ . . . . .	2: 10 (24-30 by 21-25) 26 by 22	1: 12 (24-30 by 21-27) 27 by 22	1: 16 (24-30 by 19-24) 27 by 22
Size embryo hooks, $\mu$ . .	7-8 15	7-8 3	7-8 4
Diameter scolex, $\mu$ . . .	(239-479) 315	(253-319) 288	(332-478) 406
Diameter suckers, $\mu$ . . .	11: 25 (54-96) 77	6: 16 (81-105) 93	3: 12 (93-114) 104
Diameter rostellum, $\mu$ . .	14 (72-129) 98	9 (81-122) 98	4 (111-143) 119
Age host . . . . .	4 weeks	4 weeks	8 weeks
Number cestodes in host	35 gravid 17 immature	15 gravid	7 gravid

\* Because the cestodes from these three hosts differed consistently on a number of points, they were carefully studied. In the text, the points on which these groups are separable are shown to be variously combined in an intergrading or overlapping fashion in the cestodes of other hosts. In interpreting the table, the top row of figures for any square gives the number of individuals studied and the number of measurements made for any character, with the two figures separated by a colon. The middle row, or rows, give the extremes in variation and the last row the arithmetical mean for the character.

All measurements were made without excessive flattening of the structures under consideration. Measurements of onchospheres and egg capsules were made on temporary water mounts of dissected gravid proglottids under very slight pressure. Enough measurements were made on the various structures used diagnostically to be representative of the extremes of variation of those characters and to furnish a reliable mean (See Table 1).

Numbers of rostellar hooks were determined accurately from scoleces mounted so as to present an anterior view. Measurements of rostellar hooks were made on individual hooks dissected from the scolex or on satisfactorily oriented lateral views of the hooks on mounted scoleces.

#### THE GENUS *Railletina*

The genus *Railletina* Fuhrmann, 1920, comprises numerous species found in birds and mammals. Members of the genus are characterized by a scolex armed with two rows of small hooks. Genital pores open unilaterally or alternate irregularly. The uterus breaks up into capsules containing a single or several eggs. The presence in the species here described of irregularly alternating genital pores, and egg capsules containing a single onchosphere places this form in the sub-genus *Skrjabinia*.

Previous reference to cestodes of the genus *Railletina* in prairie chickens consists only of a single citation, that of Gross (1930) in Wisconsin. These cestodes, which were identified only to genus, in this paper are assigned to *R. (S.) variabila*. The clear differentiation of this species from described species of domestic poultry cestodes would seem to suggest that *R. (S.) variabila* is indigenous to the prairie chicken.

#### *Railletina (Skrjabinia) variabila* n. sp. (Figs. 1-18)

DAVAINEIDAE Fuhrmann, 1907. *Railletina (Skrjabinia)* Fuhrmann, 1920. Possessing characters of the subgenus. Total length of the gravid strobilae 47-272 mm. Greatest width 0.6-2.8 mm. Final gravid proglottids measure 0.5-2.2 mm in length and 0.3-1.16 mm in width. The scolex is 239-479  $\mu$  in diameter. Suckers are 81-138  $\mu$  in length, 54-114  $\mu$  in diameter and are armed with 17-20 rows of small hooks, 8-10  $\mu$  in length. The rostellum is 72-133  $\mu$  in diameter and bears a crown of 240-272 hammer-shaped hooks, arranged in two rows. Those of the first row are 17-19  $\mu$  in length; hooks of the second row measure 15-16  $\mu$  in length. The oval to spherical testes number 33-82 in sexually mature proglottids of gravid strobilae and measure 24-102  $\mu$  in diameter. They may be small and scattered (Fig. 6) or large and closely packed together (Fig. 9) within the confines of the longitudinal excretory vessels. The vas deferens, exhibiting various degrees of convolution (Figs. 5-8), leads from the anterior poral side of the ovary to a muscular cirrus pouch which measures 84-213  $\mu$  by 33-119  $\mu$  and may extend from one half (Fig. 6) to all the way (Fig. 5) to the longitudinal excretory vessel. The extended cirrus (Fig. 10) is armed with minute spines and measures 33-66  $\mu$  by 10-15  $\mu$ . The genital pore varies in position from a point just anterior to the middle of the lateral margin of the proglottid to the anterior third of the margin (Figs. 6 and 9). The ejaculatory duct, usually visible as a slightly coiled tube within the cirrus pouch, in the cestodes of host 7 is club-shaped (Fig. 7). The ovary consists of two lateral wing-like extensions, deeply lobated in most individuals, and exhibits considerable variation in size within the mature proglottids in various hosts. The irregularly shaped vitelline gland usually lies in the region behind the posterior median indentation of the ovary, but in the Wisconsin specimen, mentioned later, appears behind the aporal wing of the ovary (Fig. 8). The distal portion of the vagina is dilated and has thickened walls (Figs. 13-14). The uterus breaks up into capsules each containing a single onchosphere. Capsules dissected from fixed gravid proglottids measured 60-102  $\mu$  by 42-75  $\mu$ . Onchospheres measure 24-30  $\mu$  by 19-27  $\mu$  (Fig. 15) and possess three pairs of hooks measuring 7-8  $\mu$  in length (Fig. 16).



*Habitat:* Small intestine of *Tympanuchus americanus*.

*Locality:* Jasper and Richland Counties, Illinois.

*Date:* Summers of 1936 and 1937.

*Types:* Collection of the Illinois Natural History Survey.

*R. (S.) variabila*, even with its great range of variation, is easily distinguished from the other 16 known species of the subgenus. It resembles most closely *R. (S.) centroceri* Simon, 1937, from the sage grouse in Wyoming. Through the kindness of Mr. Simon, material of *R. (S.) centroceri* was made available to the author for purposes of direct comparison. Simon (1937) states that *R. centroceri* has 198–205 rostellar hooks. This range was verified on four anterior views of mounted scoleces. Testes were said to number 63–118. Counts made on three strobilae of Simon's material gave a range of 111–158 testes per sexually mature proglottid. In view of its smaller size, more numerous rostellar hooks and significantly smaller number of testes it is believed that *R. variabila* constitutes a separate species.

It should be pointed out that Simon has erroneously included *R. (Raillietina) joyeuxi* in the subgenus *Skrjabinia*. Fuhrmann (1932) places this species in the subgenus *Raillietina*.

#### DISCUSSION OF VARIATION

Those studies on variation in cestodes which have been located in the literature are concerned largely with observations on abnormalities in form and structure and not with variation in normally formed individuals (Child, 1900 and 1902; Dobrovolny, C. G. and M. D., 1935), hence are not directly related to the present problem.

Because this collection of cestodes was at first believed to represent more than one species, most of the observations were made on those well-preserved variants which seemed to possess differences of more than specific magnitude. Consequently, those early measurements are preserved in Table 1. The discussion below will indicate the conspecific nature of the variants. The three groups mentioned in the discussion are groups 1, 2, and 3 of Table 1 and were from hosts 1, 7, and 8, respectively. It was these three groups that received the greatest study. Cestodes from other host individuals will be mentioned in the discussion as the occasion arises.

#### *Size of Strobilae*

Groups 1, 2, and 3 (Table 1) (Figs. 6, 7, 9) may be clearly separated on the basis of total length and width of complete gravid strobilae. The explanation for this appreciable difference in size of cestodes from host to host is not clear. Number of individuals and available food, age of parasite and host, and individual differences in host resistance are possible factors. It is significant that the host harboring the smallest



number of cestodes also harbored the largest. Due to size differences the 7 cestodes of host 8, the 15 cestodes of host 7, and the 52 cestodes of host 1 all filled the small intestines to an approximately equal degree.

#### *Scolex Characters*

Number and size of rostellar hooks always receive considerable attention in species determination. Descriptions of some members of this subgenus indicate little or no variation in number of rostellar hooks. *R. (S.) microcotyle* (Lopez-Neyra, 1931) is reported as having 34 hooks; *R. (S.) magnicornata* (Fuhrmann, 1909), *R. (S.) circumvallata* (Joyeux & Baer, 1936) and *R. (S.) polyuterina* (Fuhrmann, 1909) each have 200 rostellar hooks. *R. (S.) lavieri* (Joyeux & Baer, 1928) possesses 70–95; *R. (S.) ransomi* (Williams, 1931) has 500–520 and *R. (S.) cesticillus* (Joyeux & Baer, 1936) has been observed to have 200–300 in some instances and 400–500 in others. Other members of the subgenus have a range of variation from 7 to 100 hooks. Thus the literature indicates that some species of this genus may be variable with respect to number of rostellar hooks and others relatively constant. The range of 42 hooks in *R. (S.) variabila* is not unusual for this genus. Other scolex characters such as size of scolex, rostellum and suckers, and armament of the suckers offered no significantly contrasting characters within the three groups.

#### *Number and Size of Testes*

The number of testes within certain limits is considered a useful diagnostic character for members of this genus. An analysis of the number of testes for representative cestodes of the entire collection has given evidence of greater intraspecific variation than is usually encountered in cestode literature. The greatest range of variation reported for any member of this subgenus was found in *R. (S.) centroceri* (Simon, 1937). This range was 63–118, and the author, using some of Simon's material, counted as many as 158 testes in sexually mature proglottids. *R. (S.) crepidocotyle* (Joyeux & Baer, 1935) is reported as having 50–70 testes; descriptions of the other members of the subgenus indicate no range of variation greater than 10. The entire range of variation in *R. (S.) variabila* lies between 82 and 33 testes in typical mature proglottids of gravid strobilae. Non-gravid strobilae of group 1 had as low as 17 testes in the sexually mature proglottids. A glance at Table 2 will reveal minor gradations in the maximum and minimum numbers of testes for individual strobilae. It is interesting to note that the minimum numbers of testes for some strobilae exceed the maximum numbers for other strobilae.

Table 1 shows discontinuous variation in size of testes of groups 1 to 3. In cestodes of group 1 the testes are small and scattered (Fig. 6)

TABLE 2.—A study in the range of variation of number of testes within single complete strobilae, giving the maximum number for the immature proglottids, and maximum and minimum numbers in the mature proglottids for each strobila\*

No. of ind.	Maximum no. in immature proglottid	Maximum no. in mature proglottid	Minimum no. in mature proglottid	Amount of variation between extremes
1.6	63	63	42	21
1.1	66	57	38	19
1.1	55	52	37	17
1.5	51	47	33	14
1.16 imm.	58	46	28	18
1.12 imm.	53	39	26	13
1.15 imm.	48	29	17	12
7.3	..	77	62	15
7.1	76	76	58	18
7.2	80	75	56	19
8.7	82	82	60	22
8.1	77	76	62	14
8.9	68	68	57	11
8.8	65	65	50	15
Wisc.	..	56	44	12

\* The left hand column gives the number of the host and number of the cestode studied. Cestodes of hosts 1, 7, 8, representing groups 1, 2 and 3 of Table 1, were those used for this table. All specimens with the exception of those marked imm. (immature) were gravid strobilae. "Wisc." refers to the Wisconsin specimen. In some instances the minimum number of testes of cestodes in hosts 7 and 8 exceeds the maximum number for cestodes of host 1.

in contrast to the closely packed, large testes of group 3 (Fig. 9.) Without a consideration of other collections of cestodes, these two groups would undoubtedly be regarded as distinct species. Cestodes from other hosts of the same area and from a prairie chicken in Wisconsin had proglottids with 44–56 testes, the numbers characteristic of group 1, but with testes the size of those of groups 2 and 3 (Figs. 8 and 9).

#### *Size and Extent of Cirrus Pouch*

Within limits the size and the extent to which the cirrus pouch reaches medially towards the longitudinal excretory vessel have been considered as sufficiently invariable in this genus to emphasize them in description. In this study, the cirrus has been found to vary within wide limits. While its size is relatively constant within a single strobila and even in the individuals of a single host, the cirrus pouch has shown marked discontinuous variation from one host to another (Table 1).

In the cestodes of other hosts, not included in the table, cirrus and testes characters of the three groups are variously and confusingly combined. For instance, cestodes from one host had 33–42 testes, thus conforming to cestodes in group 1, but the cirrus pouch measured 170–213  $\mu$  by 87–119  $\mu$  and extended to the longitudinal excretory vessel, thereby exceeding the size of the cirrus pouch of cestodes of group 3 which are larger in length and width of strobilae. For all specimens studied, a range of 129  $\mu$  in length and 86  $\mu$  in width has been observed. A range of 50  $\mu$  in length is the greatest variation reported for any other member of the subgenus. *R. (S.) lavieri* (Joyeux & Baer, 1928) and *R. (S.)*

*crepidocotyle* (Joyeux & Baer, 1935) each exhibited this amount of variation.

The extent to which the cirrus pouch extends medially toward the longitudinal excretory vessel in sexually mature proglottids varies from a point slightly under half way to the vessel through gradations up to the vessel itself (Figs 5-9). Also in gravid proglottids, if they are narrow, the pouch may rest against the vessel (Fig. 10), whereas in wide ones, the cirrus may not reach half way to the vessel (Fig. 12). So the size and position of the cirrus pouch in this species hold limited significance as diagnostic characters.

#### *Egg Capsule and Onchosphere*

In contrast to the variation observed in the several characters discussed above, the onchospheres and egg capsules of the variants show little dissimilarity. Measurements of egg capsules and onchospheres do not change significantly in any of the groups (Table 1) and all conform to a common pattern (Fig. 16).

#### *Atypical Cestodes*

Mention should be made of a rather striking variation evident in certain strobilae of host 1, since they are sharply set off from the other members of the group. The entire strobilae are markedly smaller, and the maximum and minimum numbers of testes are below the figures for the typical members of the group. Those individuals in Table 2 marked imm. (immature) represent this group with a minimum number of 17 testes.

In these atypical individuals (Fig. 4) sexually mature proglottids are unusually narrow. The ovary remains small and undeveloped in comparison with the enlarging lobated ovary of the typical mature proglottid. Testes, in addition to being few in number, are unusually small. The final proglottids possess recognizable remains of ovary and testes, but there is no indication that the uterus is breaking up into egg capsules enclosing onchospheres. Whether these atypical members are younger and later attain the power of reproduction or for some reason are degenerate and never function reproductively can only be conjectured. Their irregularity might be associated with heavy infestation, as this four-week-old host had 52 cestodes packing the small intestine.

Young (1935), in reviewing unsolved problems of cestode structure and development, states that variation in the number of testes is related to the problem of the origin of germ cells. He had observed testes to arise from such specialized somatic tissues as muscle cells. Child (1902) observed "that reduction in size of female organs and reduction in number of testes frequently occurs in proglottids less than normal size."

*Wisconsin Specimens*

Unconformity in this species was further observed in fragments of cestodes taken from a prairie chicken in Wisconsin by Gross in 1930. This material was obtained from the Bureau of Animal Industry through the kindness of Dr. E. W. Price. These fragments, while obviously belonging to the subgenus *Skrjabinia*, combined characters of groups 1 and 3, the largest and smallest of the groups mentioned above. The one scolex obtained had hooks 16–18  $\mu$  long and, while they could not be accurately counted, were clearly numerically close to the Illinois specimens. The testes numbered 44–56 per proglottid, thus were within the range of 37–65 for group 1. However, they measured 60–93  $\mu$  in diameter, and this measurement conforms closely to the range as found in group 3, not at all to the range of group 1. The sexually mature proglottids were 1.82 mm in width, thus smaller than those of group 3, but the cirrus pouch exhibited higher maximum and average measurements, a maximum measurement of 135–180  $\mu$  by 68–81  $\mu$  and an average of 167 by 65  $\mu$ . Measurements of egg capsules and onchospheres could not be made. Thus, within a single strobila (Fig. 8) from a Wisconsin prairie chicken were found characters that might possibly have been used in separating groups 1 (Fig. 6) and 3 (Fig. 9) into separate species.

The above discussion presents what has seemed to the author an unusual amount of variation within a single species of cestode, as compared with descriptions of other described members of the subgenus *Skrjabinia*. The fact that all the hosts (Wisconsin specimen excepted) were collected within two counties and that the variations observed were similar for the cestodes of a single host, but dissimilar from one host to another, would seem to indicate that the explanation of this variation lies in a study of the relationships of parasites to their individual hosts.

## SUMMARY

1. A new species of cestode, *Railletina* (*Skrjabinia*) *variabila*, has been described. It is the first species of this genus described for the prairie chicken, *Tympanuchus americanus* (Reichenbach).

2. This species was found in ten of fourteen young prairie chickens collected in two adjacent Illinois counties. In four cases the infestations were so intense as to occlude the lumen of the small intestine.

3. Size of complete gravid strobila, number and size of testes, size and extent of cirrus pouch are characters which vary within wide limits in this species.

4. Number, size and shape of rostellar hooks, size and appearance of egg capsules, onchospheres and onchosphere hooks are relatively constant throughout the entire collection of cestodes.

5. *Railletina* (*Skrjabinia*) sp. from a prairie chicken in Wisconsin collected by Gross (1930) has been assigned to *R. (S.) variabila*.

6. Variations observed in *R. (S.) variabila* were constant for specimens within individual hosts, but noticeably different within different hosts of the same area, indicating a considerable genetic tolerance of the species to variation of the several characters mentioned. Factors responsible for variation have not been determined.

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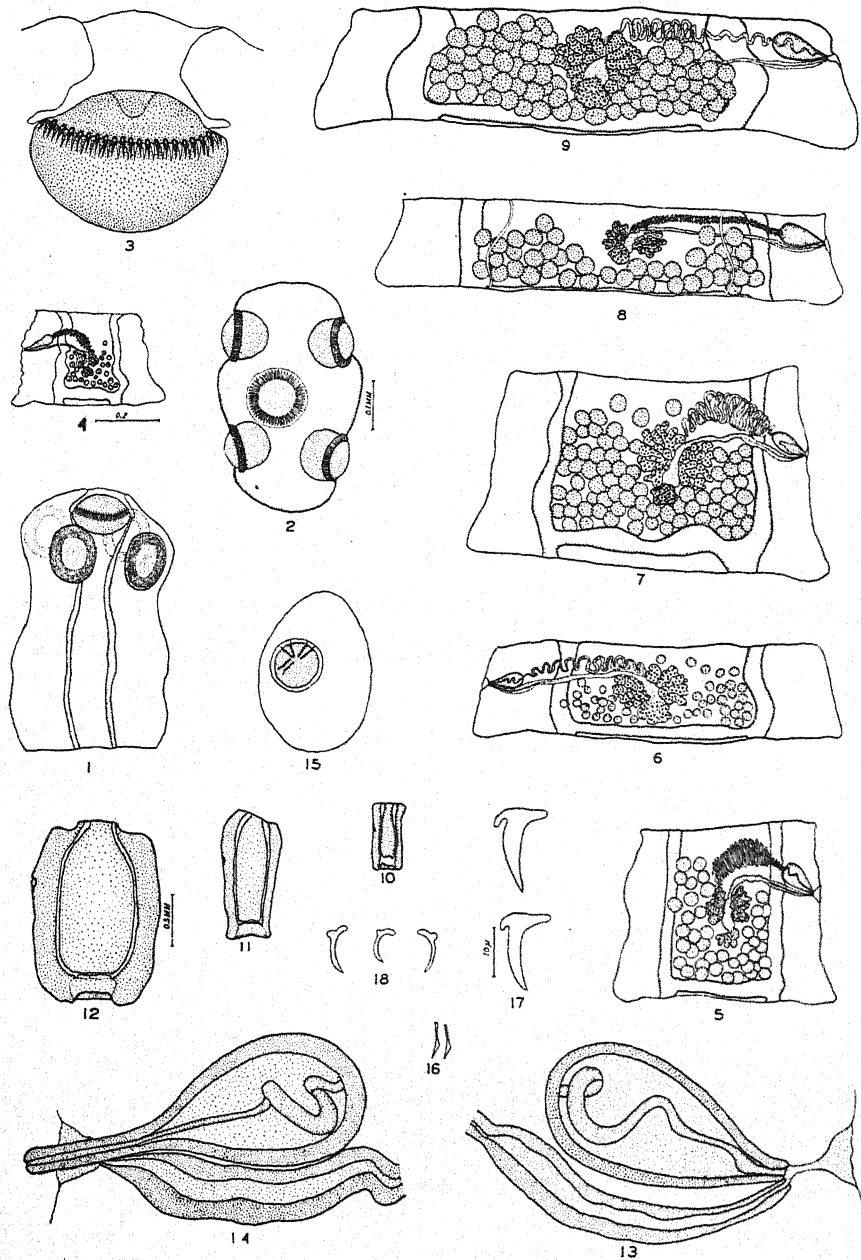
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## EXPLANATION OF FIGURES

All figures drawn with the aid of a camera lucida. Figs. 1 and 2 drawn to scale of Fig. 2; Figs. 4-9 to scale of Fig. 4; Figs. 10-12 to scale of Fig. 12; Figs. 13-18 to scale of Fig. 17. Hooks of Figs. 1-3 drawn in by hand and not representative of accurate numbers.

- FIG. 1. Lateral view of scolex, group 1.  
 FIG. 2. Anterior view of scolex, group 3.  
 FIG. 3. Lateral view of rostellum, showing arrangement of hooks.  
 FIG. 4. Atypical proglottid group 1, drawn to same scale as Figs. 5-9.  
 FIG. 5. Proglottid combining some features of Figs. 6 and 7.  
 FIG. 6. Sexually mature proglottid, group 1.  
 FIG. 7. Sexually mature proglottid, group 2.  
 FIG. 8. Sexually mature proglottid, from Wisconsin specimen.  
 FIG. 9. Sexually mature proglottid, group 3.  
 FIGS. 10, 11, 12. Final gravid proglottids of groups 1, 2, and 3, respectively, drawn to same scale.  
 FIG. 13. Cirrus pouch-vagina complex, cirrus unextruded.  
 FIG. 14. Cirrus pouch-vagina complex, spined cirrus extruded.  
 FIG. 15. Egg capsule showing onchosphere and inner envelope.  
 FIG. 16. Onchosphere hooks.  
 FIG. 17. Rostellar hooks.  
 FIG. 18. Acetabular hooks.





*Raillietina (Skrjabinia) variabila*

## RECOVERY OF *TRICHINELLA SPIRALIS* LARVAE IN EARLY STAGES OF INFECTION

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Although the life cycle of *Trichinella spiralis* is known in a general way, many of the details remain obscure, due to the inadequate methods employed in their study. To cite one instance, the early phases of the life cycle of *Trichinella* have not been investigated because the methods available for obtaining larvae in later stages of infection have not been of use in recovering the young worms. Leuckart (1) and his contemporaries used a method of scraping and chopping the meat and washing the larvae out for recovery of worms in all stages of infection. Ransom (2) devised the method of pepsin digestion, and the workers in the field immediately took up this cleaner recovery technique. However, since the young worms cannot withstand this digestive treatment (McCoy, 3), no publications have appeared on this phase of trichina investigation since that time. This present study was undertaken for the purpose of establishing a quantitative method for the recovery of larvae in the early stages of infection.

### MATERIALS AND METHOD

The rats used were albino and hooded laboratory stock, between 11 and 20 weeks old. They were fed on a diet of dog biscuits and water, and kept under the same conditions. The *Trichinella* larvae used for infection were obtained from rats, infected eight weeks or more, by the method of digestion described by Ransom. After having previously been starved for 24 hours, each group of rats was infected at the same time with a dose of 30 larvae per gram body weight introduced into the stomach with a pipette. When the predetermined time of infection had elapsed, each rat was killed and the larvae recovered as described below:

1. Strip the skin and eviscerate the rat.
2. Grind the meat through a fine mesh grinder. Do not allow the meat to become dry at any time from this point on.
3. Separate ground meat into parts of not more than 25 grams each. Larger masses necessitate many washings to recover all the larvae.
4. Place each 25 grams (or less) of meat into a 500 cc Erlenmeyer flask, and add 60 cc 0.9% saline for each 10 grams of meat. Introduce glass beads into the flask and shake vigorously for 3 to 4 minutes to further break up the muscle fibers.
5. Filter off all the liquid through a 60 mesh screen placed in a Buchner funnel in a suction flask. To keep the meat in the flask while pouring the fluid off,

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the opening of the flask should be covered with cheesecloth. Any meat adhering to the cloth can be washed back by pouring saline over the cloth into the flask. Discard each piece of cheesecloth after being used once.

6. Wash meat by adding 30 cc 0.9% saline per 10 grams meat and shake the mixture for 2 to 3 minutes. Filter off the liquid and add to the first filtrate.

7. Add 60 cc of 0.9% saline for each 10 grams of meat and connect the flask to a compressed air hose. Force air through the mixture vigorously for one hour at room temperature (22° C). Higher temperatures cause a froth to form, making it more difficult to wash all the larvae out of the meat.

8. Filter off the liquid through a 60 mesh screen and add to the first filtrate.

9. Wash meat *twice* as described under (6). Add all liquid to the first filtrate.

10. Centrifuge the total filtrate suspension to concentrate the larvae. The supernatant fluid should be examined for larvae before being discarded. The concentrated larvae can be kept indefinitely by adding some 5% formalin (5 cc) and storing at 5° C.

11. Make larvae counts by drawing 0.1 cc samples from the larvae concentrate made up to known volume (McCoy, 4) and count under the low power of the microscope on a ruled glass slide.

In working out this method, the meat was washed 10 to 15 times after being subjected to the compressed air treatment, and only in three cases were any additional larvae found after the first washing; 50 larvae were found in one case on the 14th day after infection, 10 larvae on the 16th day after infection, and 100 larvae on the 17th day after infection. The second washing was therefore added to the technique to insure recovery of all the larvae.

#### EXPERIMENTS AND RESULTS

Each group consisted of ten rats infected at the same time with larvae from the same source. The first rat was killed five days after infection, the second six days later, and so on through fourteen days. Two groups of rats were so treated. A third group consisted of five rats. The first was killed 5½ days after infection, the second 6 days after infection, the third 8 days after infection, the fourth on the 9th day and the fifth 11 days after infection. The infective dose consisted of 30 larvae per gram body weight administered per os. No larvae were recovered five and a half days after infection. On the 6th day, as many as 50,000 worms were recovered, ranging in size from 95 to 125 microns in length by 5 microns wide, which is the size of larvae forced from a gravid female. From the 7th to the 11th day inclusive, the increase in numbers of larvae is marked; 135,000 being recovered on the 7th day, 151,000 on the 8th day, 221,000 on the 9th day, 316,000 on the 10th day, and 350,000 on the 11th day. The number recovered thereafter remained relatively constant; 415,000 on the 12th day, 435,750 on the 13th day, and 415,000 on the 14th day. Larvae-larvae ratio calculations were made on the basis of the number of larvae fed and the number of larvae recovered each day. These results are summarized in Table 1, and graphically in Fig. 1. Daily measurements of the larvae indicate that the larvae of 14 days infection reach a maximum size of 375 microns in length and 22.5 microns in width. How-

ever, the size and growth relationship of the young larvae are being studied at present and will be summed up in another paper.

The young larvae, up to 12 days after infection, could be recovered alive when the following modification of the maceration method was used:

The entire muscle portion of the rat, finely ground, was placed in 0.9% saline in a flask (see Nos. 1 and 2 under Method) and shaken gently. Then the liquid was filtered off through a fine mesh screen (see No. 5 under Method). The filtrate contains the living larvae. This

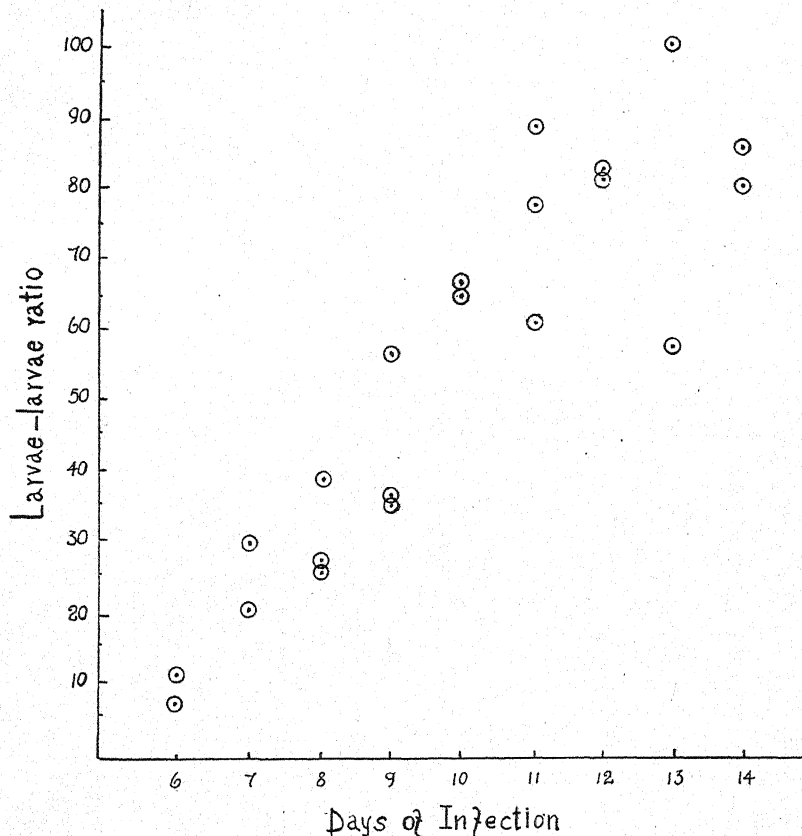


FIG. 1. A graphic representation of the daily larvae-larvae ratio (number of larvae recovered divided by the number of larvae fed).

modification of the maceration method is of use only in obtaining the young larvae alive. The number of larvae recovered in this way is in no sense a true count of the total number of larvae contained in the muscles of the rat. From the 12th day after infection on, many of the young worms will remain alive even after having been put through the complete process of maceration, as outlined under Method.

A fourth group of rats was used to find how long after infection the method could be used efficiently. One rat was killed 16 days after infec-

TABLE 1.—Data from Groups 1, 2, and 3

Rat	Days infected	Weight at infection, grams	No. larvae fed	Weight when killed, grams	No. larvae recovered	Larvae-larvae ratio
*I—1	5	188	5640	143	0	...
II—11	5	187	5630	168	0	...
III—22	5½	134	4020	...	0	...
I—2	6	174	5220	133	0	...
II—12	6	174	5200	143	32,200	6.2
III—30	6	174	4500	119	50,500	11.2
I—3	7	188	5640	132	120,000	21.3
II—13	7	169	5060	137	149,000	29.4
I—4	8	182	5460	131	145,000	26.6
II—14	8	184	5500	160	151,875	27.6
III—31	8	135	4050	124	155,000	38.3
I—5	9	226	6780	159	237,500	35.0
II—15	9	161	4840	138	172,500	35.6
III—27	9	150	4500	126	253,000	56.2
I—6	10	165	4900	102	330,000	67.3
II—16	10	161	4830	145	317,272	65.7
I—7	11	159	4770	117	295,000	61.8
II—17	11	168	5050	144	450,000	89.1
III—36	11	150	4500	126	350,750	77.9
I—8	12	167	5010	126	415,000	82.8
II—18	12	165	4960	143	400,000	80.6
I—9	13	240	7200	151	414,750	57.6
II—19	13	153	4570	122	458,750	100.4
I—10	14	169	5070	151	430,250	84.9
II—20	14	169	5070	134	401,250	79.1

\* The Roman numerals and Arabic numbers (e.g. II—19) refer to the numbers of individual experiments.

tion, another at 17 days, another at 21 days and the last at 28 days. As previously noted, larvae were recovered after the first washing on the 14th, 16th, and 17th days. This seemed to indicate that there was a time after which this method would be less efficient. By the 21st day, the maceration method was of no value in recovering the larvae. The digestion method (80:1 pepsin dilution) was efficient from this point on. Table 2 summarizes the results of this group.

Some rats yielded results far out of line with the majority of cases. Possible causes for these variations will be discussed later.

TABLE 2.—Data from Group 4

Rat	Days infected	Weight at infection, grams	No. larvae fed	Weight when killed, grams	No. larvae recovered	Larvae-larvae ratio
*IV—25	16	170	5100	173	117,000	22.9
IV—29	17	150	4500	143	152,250	33.8
IV—34	21	142	4240	140	380,750 (total)	89.8
					maceration : 236,250	
					digestion : 144,500	
IV—35	28	146	4380	152	263,750** (total)	60.2

\* The Roman numerals and Arabic numbers (e.g. IV—29) refer to the numbers of individual experiments.

\*\* The ground meat from this rat was divided into two equal parts: A and B. Part A was first macerated and then digested. Part B was digested only. Part A. Maceration: 17,000 larvae recovered; digestion: 112,500 larvae recovered. Part B. Digestion: 134,250 larvae recovered.



## DISCUSSION

The maceration method utilized here is based on the consideration that the young larvae can be recovered by mechanical agitation of finely ground meat because they do not become encapsulated in the muscle sheath until at least four weeks after infection.

Most observers are agreed upon the fact that the first larvae appear in the host tissue about the fifth or sixth day after infection (Leuckart (1), Virchow (5), and others). In this work, no larvae were recovered until the sixth day. The duration of larval production and larval migration has been estimated by various authors at from six to twenty-five days (Leuckart (1), et al; Augustine (6)). According to Leuckart, the greatest migration takes place on the 9th and 10th days and the maximum invasion of muscle occurs between the 12th and 15th days. Augustine places the maximum invasion on the 10th day. This present work seems to indicate that the deposition of larvae, once it is begun, is a rapid and constant process, occurring over a period of six days. Larval migration seems to be of very short duration, and penetration of the host tissue seemingly takes place very shortly after the larvae have been released. Since the number of larvae recovered after the 12th day does not increase, it seems correct from these experiments, to set the duration of larval production and migration at from the 6th to the 12th days.

The small intestine of each rat was examined for the presence of adult worms. Up to the 16th day, adults were found. On the 16th day and thereafter, no adults were found in the intestine. This corresponds exactly with the findings of Christenson (10) and Gursch (7).

Although most of the data checks with a fair degree of accuracy, variations do occur. These variations seem to be of two kinds: one, wherein the number and size of the larvae recovered on a particular day are smaller than that ordinarily found; and two, wherein the number of larvae recovered on a particular day is less than might be expected, based on previous experiments, although the size of the larvae corresponds with the size of those usually found on that day. The former type of variation will be discussed in a subsequent paper on size and growth relationships of the larvae.

The latter type of variation is exemplified particularly in Group IV. Gursch (7), working in this laboratory, using the same stock of rats, the same source of larvae and the same infection dose, found similar variations. In one case, in a 12 day infection, he could recover only 15 per cent of the original dose from the intestine, whereas 42 per cent recovery is usual. Furthermore, in another rat, examined 10 hours after infection, he found 37 per cent of the larvae still in the stomach, whereas all the other cases showed less than one per cent. According to Glazier (8), Flint states that the liberation of trichinae in the stomach corresponds

with the time required for solution by the gastric juice of the albuminous substances of the muscle, which takes from one and a half to five hours. Professor Wedl, in a report to the Medical Society of Vienna in 1867, further states that in vitro, free trichinae are acted on and killed by gastric juice, while it only serves to dissolve the capsule in other cases and thus set free the worm. From this, one might postulate that worms, previously digested out and fed, and then remaining for an additional ten hours in the stomach of the host would take little, if any part in producing an infection.

McCoy (9) was able to demonstrate that, in immunized rats, larvae fed were quickly passed in the feces. It is conceivable that this might occur in rats showing a natural immunity. Thus, the retention of larvae in the stomach, and the passing out of larvae in the feces before producing an infection could account for a low infection in non-immunized rats.

#### SUMMARY

1. Young *Trichinella* larvae, in infections of from six days to twenty-one days, can be recovered from rat tissue by a technique of maceration. This technique separates the larvae from host tissue by physical means, without resort to chemicals, such as pepsin, which destroy the young worms.

2. When an infective dose of approximately 5000 larvae was used, the first evidence of larvae in the rat tissue was obtained about six days after infection.

3. The numbers of larvae released into the rat tissue increase rapidly from the sixth to the eleventh day. By the twelfth day, the great majority of larvae have apparently been released. The numbers recovered remain fairly constant from the twelfth day onward.

4. The size of the larvae recovered from rat tissue on the sixth day is the same as the size of larvae squeezed from a gravid female.

5. Extreme variations from the normal (majority of cases) in the number of larvae recovered may be accounted for by the fact that, in some instances, the larvae fed may have remained in the stomach for abnormally long periods of time and died before passing into the small intestine; and the possibility of their being passed in the feces before taking part in producing an infection.

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## DISCOVERY OF HUMAN HEARTWORM INFECTION IN NEW ORLEANS\*

ERNEST CARROLL FAUST, E. PERRY THOMAS, AND JACK JONES

Heartworm infection in domestic dogs is prevalent throughout Southern Europe, China, Japan, the Netherlands Indies, Fiji, New Caledonia, Australia, Hawaii, and the warmer regions of North and South America. In some of these areas the incidence in dogs over one year of age is as high as 30 to 50 per cent. The etiological agent of this infection is *Dirofilaria immitis* (Leidy, 1856). This same species of filaria has also been recorded from the dingo (*Canis dingo*) in Australia; from *C. brachyurus* in Brazil; from the wolf (*Canis* sp.) in Japan; from the timber wolf (*C. floridanus*) near New Orleans, La.; from the fox (*Vulpes vulpes*) in North China; from the domestic cat (*Felis catus domestica*) in Virginia (U. S. A.), Dutch Guiana and North China; from the tiger (*F. tigris*) in French Indo-China; from *F. tigris sondiaca* in the Netherlands Indies, and from the jaguar (*F. onca*) in Surinam. Moreover, the specimens of *Dirofilaria* obtained from the common seal (*Phoca vitulina*), the crested seal (*Stenmatopus cristatus*) and the California sea-lion (*Zalophus californianus*) are apparently indistinguishable morphologically from the dog heartworm (Faust, 1937). Another species of heartworm (*D. pongoi*) has been described by Vogel and Vogelsang (1930) from the Borneo orang-outang (*Pongo pygmaeus*), and still another species (*D. indica*) by Chakravarty (1936) from a Calcutta dog.

With the possible exception of a few observations made in Tokyo by Tokishige (cited in Blanchard, 1895), these worms have been recovered from the chambers of the right heart, the inferior and superior vena cava, and the pulmonary artery, or, less frequently, have migrated from the pulmonary artery into the respiratory tree. Tokishige reported 82 heartworm infections in dogs, of which one occurred in the left heart, one in the posterior (abdominal?) aorta, and one in the crural artery, while in one instance the worms had perforated the interauricular septum.

In 1887 de Magalhães reported the discovery of a single male and a single female filaria in the left ventricle of a male child from Rio de Janeiro. These worms were studied by Blanchard (1896), who stated

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that de Magalhães' description was accurate but recognized that the worms were specifically different from the dog heartworm and named them *magalhãesi* in honor of their discoverer.

The heart filarias all belong to the subgenus *Dirofilaria* Faust, 1937, as distinguished from the species of *Dirofilaria* living in the cutaneous and subcutaneous tissues (subgenus *Nochtiella* Faust, 1937).

#### PRESENTATION OF DATA

The present report concerns a second case of human heartworm infection, from an aged negress, native and life-time resident of New Orleans, Louisiana.

The woman had never gone into the country for a visit or to work. Her only absence from the city had occurred more than twenty years ago, when she was placed in a government hospital for treatment of drug addiction. She died at the age of 73, probably as a result of acute toxic nephritis. The body did not come to autopsy but was embalmed and later was utilized for dissection. In the abdominal cavity there was a peritoneal abscess around the cecum, extending along its medial and lateral sides; it was adequately walled-off by the omentum and small intestine. There was no evidence of edema or abdominal ascites. The kidneys presented gross evidence of acute toxic nephritis. The pleura, lungs and heart showed no gross pathology.

On dissection of the inferior vena cava a stiff, creamy-white thread was discovered projecting out of a blood clot. After the object had been washed and placed in formalin it was evident that it was no artefact. Examination with the naked eye and with a hand lens suggested that it was a male filaria, especially in view of the characteristic corkscrew coiling of its more attenuated end. Search for a female worm was unsuccessful and examination of a portion of the blood clot for microfilariae was negative. It was then concluded that the infection consisted of a single male worm, which was cleared in glycerin and was found to be a specimen of *Dirofilaria*. Additional study was then undertaken to discover whether (1) it was *Dirofilaria immitis*, which is quite common in dogs in the New Orleans area, (2) *D. magalhãesi*, or (3) a new species of *Dirofilaria*.

#### DESCRIPTION OF THE WORM

The worm is a mature male. It is creamy white, delicate, filiform and wiry (Figs. 1, 2A). The anterior end is stiff and bluntly rounded. The posterior end is attenuate and exhibits a typical cork-screw coiling through at least three complete spirals. The total length of the filaria is 120 mm, the greatest diameter, 0.52 mm, and the diameter of the head, 0.41 mm. The integument is thick and is transversely striated, especially on the inner curvature of the tightly coiled posterior end. Second only

to the posterior spiralling, the most conspicuous feature of the worm is the relatively long ala on each side of the posterior extremity (Fig. 2D), with a length of 0.55 mm and a maximum breadth of 48 microns. Under low magnification of the uncompressed caudal end the 4 pairs of large preanal papillae were readily observed, while the 4 pairs of smaller postanal papillae were detected with considerable difficulty. For more detailed examination the posterior-most one mm was separated from the major portion of the worm with a sharp scalpel, mounted on a slide under a coverglass and studied under a magnification of 500 diameters (i.e., 4 mm objective and paired  $\times 8$  oculars).

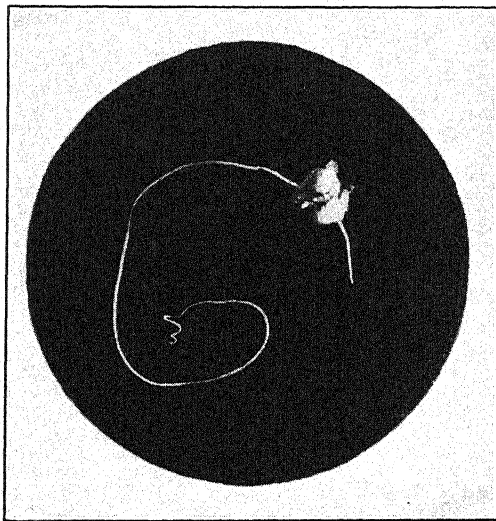


FIG. 1. Photograph of entire male *Dirofilaria louisianensis* n. sp., natural size.

As observed either from the ventral or the lateral aspect (Fig. 2D, E) the caudal extremity showed clearly the 4 pairs of large, obovoidal, symmetrically arranged preanal papillae, approximately equal in size, each with a delicate central duct which apparently arises from a basal gland and opens externally through a pore. The 4 pairs of symmetrically arranged postanal papillae were considerably smaller. The two median pairs were structurally like the preanal papillae, but were only about half their size. The pair of papillae immediately posterior to the cloacal opening was even smaller, semi-stalked and its two members close together. Each papilla of the posteriormost pair resembled a delicate shoe-button on a moderately long, delicate stalk. All of these papillae were found to be smooth surfaced.

Cephalic papillae were difficult to detect but careful examination indicated that there were ten in all, consisting of four pairs of submedian

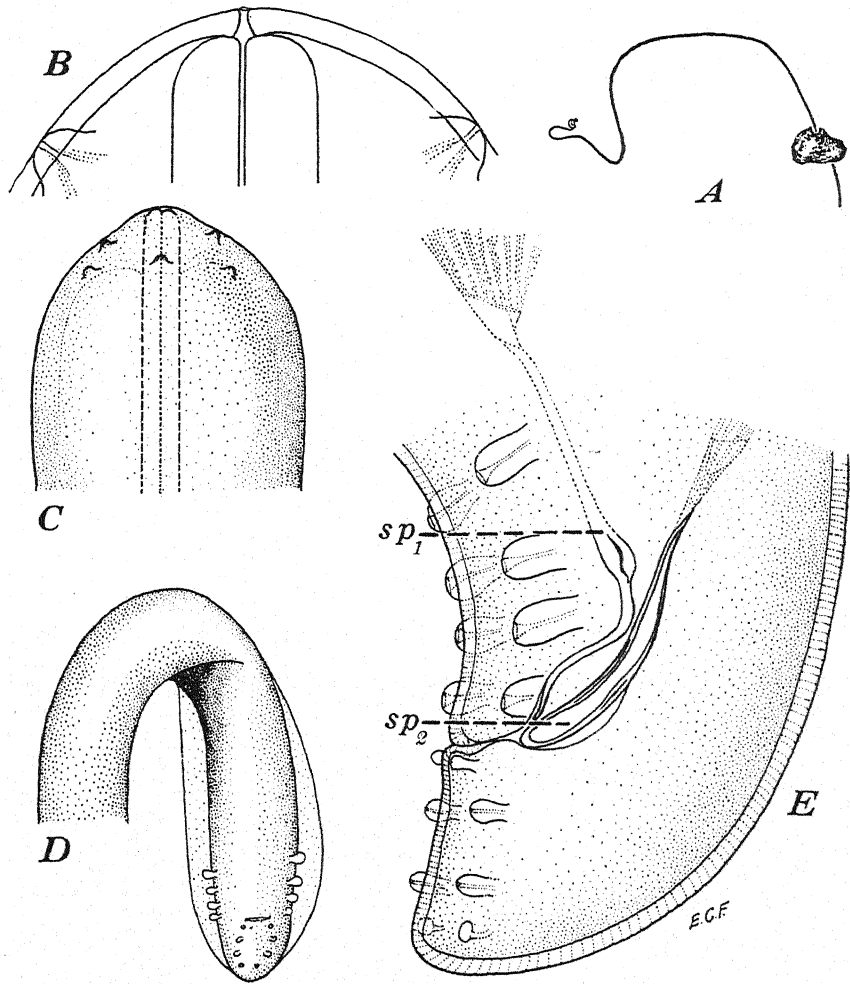


FIG. 2. *A*, camera lucida sketch of entire worm, with adherent portion of blood clot near anterior end,  $\times 2/3$ ; *B*, extreme anterior end of worm, lateral view, showing thickness of integument, two of the submedian papillae, proximal end of esophagus and minute buccal cavity,  $\times 333$ ; *C*, anterior end of worm, lateral view, showing contour of cephalic end, four submedian and one labial papillae, and portion of the esophagus,  $\times 85$ ; *D*, posterior end of worm, ventral view, uncompressed, showing caudal alae, cloacal cleft, 4 preanal papillae and 4 postanal papillae,  $\times 85$ ; *E*, posterior end of worm, nearly lateral view, slightly compressed, showing paired preanal and postanal papillae, long, narrow, left spicule ( $sp_1$ ) and shorter, relatively broad, right spicule ( $sp_2$ ),  $\times 333$ .

papillae and one pair of lateral papillae (Fig. 2*C*). This conforms to the pattern presented by Chitwood and Chitwood (1938) for *Dirofilaria immitis*, which Desportes (1940), apparently unaware of the Chitwoods' data, identifies as a constant diagnostic character of the genus *Dirofilaria*.

The copulatory spicules (Fig. 2*E*,  $sp_1$ ,  $sp_2$ ) varied specifically from

TABLE 1.—Comparative data on species of *Dirofilaria* (subgenus *Dirofilaria*) (*Males only*)

	<i>D. magalhãesii</i> (from Brazilian boy)	<i>D. magalhãesii</i> (from Brazilian boy)	<i>D. louisianensis</i> n. sp. (from New Orleans negro)	<i>D. immitis</i> (from dogs)	<i>D. indica</i> (from Calcutta dog)	<i>D. pongoi</i> (from Borneo orang-outang)
Length, mm . . . . .	83	83	120	120-200	130-170	107-125
Breadth, mm . . . . .	0.28-0.40	0.407	0.52	0.6-0.9	0.7	0.33
Distance of cleavea from caudal tip, mm . . . . .	0.11	0.11	0.33	0.075-0.12	0.110	0.095
Spicules, mm . . . . .	Apparently single, 0.17 long	Shorter, 0.23; longer, undoubt- edly present (?)	Shorter, 0.150; longer, 0.196	Shorter, 0.2- 0.226; longer, 0.3-0.355	Shorter, 0.165; longer, 0.385	Shorter, 0.18- 0.193; longer, 0.300-0.312
Papillae: preanal . . . . .	4 pairs, large, "villous," symmetrical	4 pairs, large, "villous," symmetrical	4 pairs, large, smooth, symmetrical	3-4 pairs, large, smooth, asymmetrical	4 pairs, large, "granular," bifidly digitate; one small papilla, median ventral; one pair, slender, adanal	4 pairs, large, club-shaped, having appear- ance of "pine cones"
" postanal . . . . .	4 pairs, "villous," symmetrical; last 2 pairs smaller	4 pairs, "villous," symmetrical; last 2 pairs smaller	4 pairs, smooth, symmetrical; first and last pairs smaller	1 pair large, smooth, asym- metrical; 3-4 pairs, smooth, very small	9 pairs, some pedunculate, some sessile, varying in size but smaller than preanals	3-4 pairs, small digitate to con- ical; 1 pair very small, close to- gether, immedi- ately post-anal
Remarks . . . . .	Based on study of worm by Blanchard (1896)	Based on French résumé sent by de Magalhães to von Linstow (1900)	Based on study of worm by pres- ent authors	Based on data of Fülleborn (1912), Vogel (1927), Faust (1937)	Based on study of worms by Chakravarty (1936)	Based on study of worms by Vogel and Vogel- sang (1930)

those of described species of *Dirofilaria*. The right spicule of this worm (the shorter one), like that of other species of the subgenus, was seen to be fashioned as a moderately long, shallow, gracefully curved scoop, with a rounded distal end. On first examination its proximal (i.e. attachment) end appeared to be distinctly pointed, but more careful study revealed a nearly transparent, inverted triangular extension which constituted the actual internal shank of this spicule. It had a length measurement of approximately 150 microns, had ample skeletal support except at the proximal end, and was readily seen in the cleared specimen. The left spicule (the longer one) was more delicate, practically hyaline, and easily overlooked. It was not as sharply pointed at its distal end as that of *D. immitis* or *D. pongoi*, was curved on itself in its distal third to form a drawn-out figure S, had a distinct thickening at the level of flexure and a narrow shaft from this point to its proximal end, where it flattened out at its site of attachment. It had a length measurement of 196 microns.

The more important characters of this worm, together with comparable data from *D. magalhães*, *D. immitis*, *D. indica* and *D. pongoi* are summarized on Table 1.

There is no evidence associating this worm with any pathological procession in the host.

#### DISCUSSION

The discovery of a second isolated heartworm infection in man from an area far removed from that in which de Magalhães discovered the first human infection more than a half century ago indicates that man is susceptible to heartworm infection. However, since the human heart is almost invariably opened at autopsy, filarias, if present in the chambers of the heart or the great vessels leading into or from it, would probably not be overlooked, nor would they be diagnosed as artefacts. Thus, the absence of additional cases of human infection suggests that human heartworm infection is indeed rare.

If de Magalhães' worm and that from New Orleans were diagnosable as *Dirofilaria immitis*, an explanation of the two isolated human infections would be readily afforded, namely that this worm, which is so common a parasite of dogs, may, under appropriate circumstances, become a parasite of man. However, the present morphological criteria for species differentiation of filarioid worms require that the specimens obtained from the two human subjects be regarded as distinct from *D. immitis*, *D. indica* and *D. pongoi*, as well as from one another. Although it is apparent that parasites rarely reported from a particular host must have adequate reservoirs to maintain their life cycles, there is neither proof nor suggestive evidence indicating what these reservoirs are in the case of the two filarias recovered from the human heart.



The dilemma which results may be summarized as follows. From a standpoint of pure logic de Magalhães' worm and that from New Orleans should not be considered as distinct species, but until a broader species concept of filarioid worms is provided they must be designated as such.

*Dirofilaria louisianensis* n. sp.—with the characters of the genus *Dirofilaria*, subgenus *Dirofilaria*. Only a single male worm known, having a length of 120 mm, a greatest breadth of 0.52 mm and a distance from the cloacal cleft to the caudal extremity of 0.33 mm; with caudal alae 0.55 mm long and 48 microns wide; with 4 pairs of large, smooth, obovoidal, symmetrically arranged preanal papillae, approximately equal in size; with 4 pairs of relatively large, smooth, obovoidal, symmetrically arranged postanal papillae, of which the median two pairs are the larger and are similar in shape and structure to the preanal papillae, the first and fourth pairs are much smaller, the fourth pair having a shoe-button head surmounting a moderately long delicate stem; with a shorter (right) spicule, easily seen, measuring 0.150 mm in length, rounded at its distal end, with a nearly transparent proximal end; with the longer (left) spicule, delicate and nearly transparent so that it is not easily detected, measuring 0.196 mm in length, with an S-curved distal portion, a thickened flexure and a thin, flattened proximal end.

*Habitat*: New Orleans, La., U. S. A.

*Host*: Elderly negress, native and life-time resident of New Orleans.

*Location of parasite in host*: Inferior vena cava.

The specimen is deposited in the Faust Helminthological Collection.

#### SUMMARY

Dissection of the body of an aged negress, a native and life-time resident of New Orleans, La., has resulted in the recovery of a single adult male filaria from a blood clot in the inferior vena cava. The failure to find any females or microfilariae suggests that the male was the only worm that had developed. There was no evidence indicating that the heartworm infection had produced any pathology.

The measurements of this heartworm, together with the character of the preanal and postanal papillae and the structure of the copulatory spicules, indicate that on morphological grounds the worm is specifically different from the common heartworm of dogs (*Dirofilaria immitis*), from *D. indica*, from *D. pongoi* and from *D. magalhães*i, the only previously reported heartworms of mammals. Based on these criteria this worm is designated as *Dirofilaria louisianensis* n. sp.

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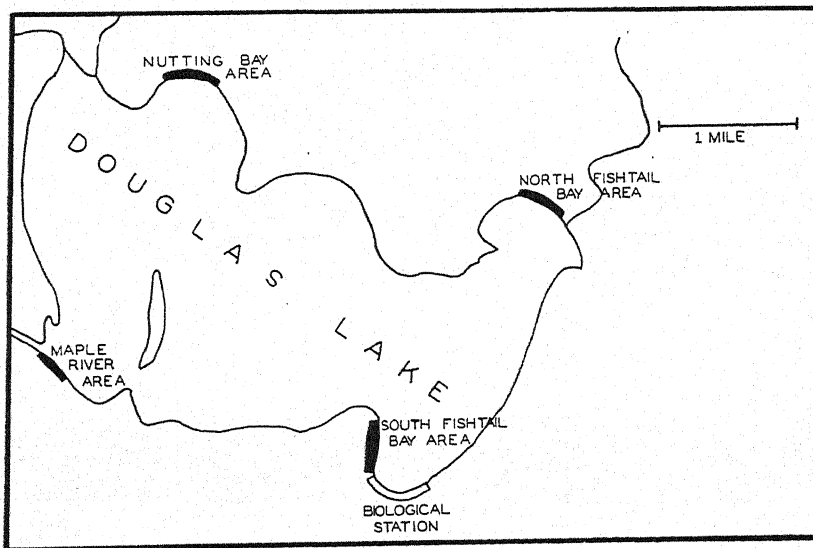
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# LARVAL TREMATODE INFECTION IN JUVENILES AND ADULTS OF *PHYSA PARKERI* CURRIER<sup>1</sup>

W. W. CORT, LOUIS OLIVIER AND D. B. McMULLEN

## INTRODUCTION

Studies on the ecological relations of larval trematode infections in *Stagnicola emarginata angulata* (Sowerby) have been carried out during the last five summers at the University of Michigan Biological Station (see Cort, McMullen, and Brackett, 1937; Cort, McMullen, Olivier, and Brackett, 1940a). One of the most interesting parts of this work has been the comparison of infections in juvenile and adult snails. In the summers of 1938 and 1939 the ecological studies were extended to include the larval trematode infections of juveniles and adults of *Physa parkeri* Currier, a very large beach snail, and, in addition, its life cycle was worked out. Two preliminary publications have already been made on certain phases of this work (Cort, McMullen, and Olivier, 1938; Cort, McMullen, Olivier, and Brackett, 1940b). In this paper all the data obtained on the life cycle of *P. parkeri* and on its larval trematode infections are presented.



MAP 1. Map of Douglas Lake showing the location of the four areas from which the collections of *P. parkeri* were made.

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<sup>1</sup> A contribution from the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

The collections of *P. parkeri* for this study were made from four areas on Douglas Lake (see Map 1). Of these, the North Fishtail Bay, Nutting Bay, and Maple River areas were also used in the studies of *S. emarginata angulata*. The collections of *P. parkeri* from the Maple River area, however, covered a much larger stretch of beach extending almost a half mile south and east of the source of Maple River. Here the beach is wide, the water is shallow, the soil contains much organic material, and there are many reed beds. In addition, collections were taken from the South Fishtail Bay area, which extends north about half a mile from the western limit of the Biological Station. The beach here varies considerably in width, but is narrow for most of the way. These four areas are among the best places for collecting *P. parkeri* in the whole region.

The methods used in this study were the same as those described by Cort et al (1940a).

#### LIFE CYCLE OF *Physa parkeri*

*Physa parkeri* is by far the largest species of this genus in the Douglas Lake region. Our measurements of adults (Table 1) show a range of shell length from 13–25 mm with the mode at about 20 mm.<sup>2</sup> These snails are very delicate and die quickly when brought into the laboratory unless handled very carefully and kept in running water. The shells, both of the juveniles and adults, are thin and easily broken in handling. This species rarely occurs in large numbers and appears to be easily affected by adverse conditions in its natural environment. *P. parkeri* has only been found on the shores of Douglas Lake. It is more restricted in its habitat than *S. emarginata angulata*, although it is sometimes found along stretches of beach where that variety is absent.

In the summer of 1939 we were able to make a few observations on the egg-laying habits of *P. parkeri*. From June 17 to 20 several egg masses were found and juveniles began to hatch from one of these on June 22. On June 20, July 4, and July 5 to 11, eggs were laid in the laboratory containers by snails brought in from three different areas. From four egg masses laid on June 20, juveniles hatched in 16 and 17 days, and during a warmer period (July 4 to 14) they hatched in from 8 to 10 days. The egg masses of *P. parkeri* are somewhat variable in size and shape and are usually larger than those of *S. emarginata angulata*. They are elongate, curved masses of jelly for the most part about 12 to 18 mm in length and are rather soft and easily broken. One unusually large mass was almost 50 mm long by about 12 mm wide. The differences in size depend on the number of eggs, which varied in 22 counts from 5 to 161. Most of the masses, however, contained from

<sup>2</sup> For the description of the methods used in making the measurements see Cort et al, 1940a, p. 35.



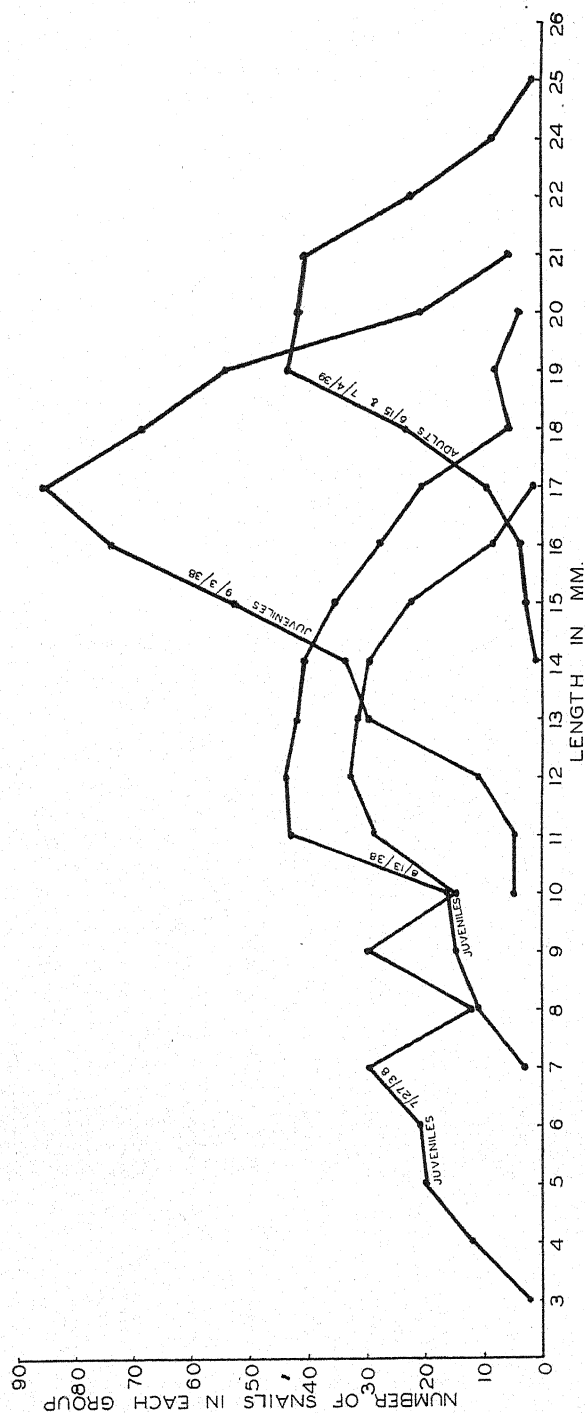


40 to 80 eggs. The newly hatched juveniles measured about 1 mm in length.

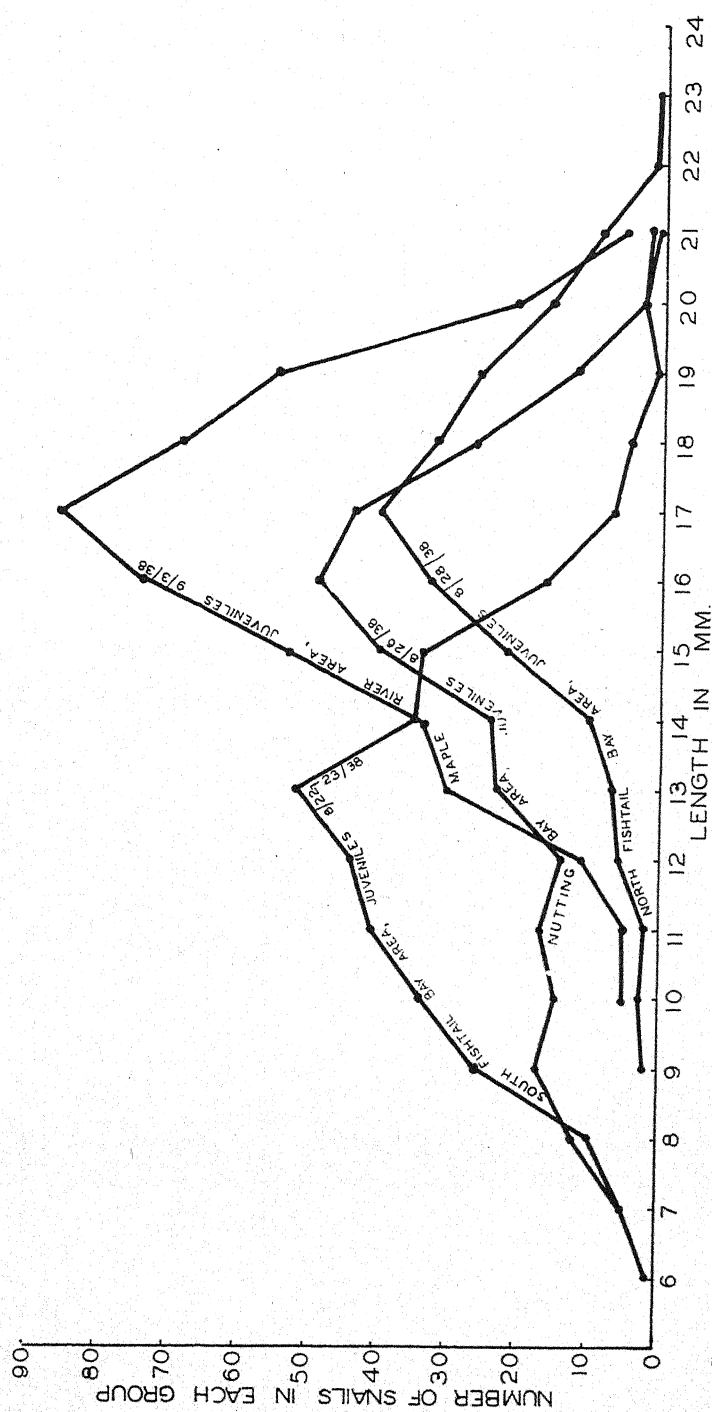
In the summer of 1938 we started to collect juveniles of *P. parkeri*. Unfortunately we have only a few records of early summer collections. On July 11 in making a collection of adults from the Maple River area we accidentally picked up five juveniles of this species from 11 to 14 mm in length. This suggests that on that particular beach some juveniles must have started to hatch in the first half of June or even earlier. Our best series of measurements of juveniles of *P. parkeri* was taken from specimens collected in the Maple River area on July 27, August 13, and September 3, 1938 (Table 1 and Graph 1). The 300 juveniles collected on July 27 had a surprising range of variation in length (3 to 17 mm) which overlapped the adult size. It seems probable that the smallest of these juveniles had hatched from eggs laid on the beach early in July. The considerable proportion of them that had reached a large size suggests that eggs of this species may be laid on the beaches somewhat earlier than those of *S. emarginata angulata*. By August 13 they had increased considerably in size, with more than half reaching a length greater than the minimum for adults (13 mm). None under 7 mm was present in this collection and there was a greater tendency for concentration of the length measurements around the mode. By September 3 all except a very few were in the adult size range. The minimum lengths in the three collections of juveniles shown in Graph 1 suggest that few eggs were being laid on this beach after the first week in July and that few if any snails were hatching after the middle of that month. We interpret this and the other evidence on the breeding season of *P. parkeri* as indicating that the eggs are laid in June and early July.

Late August collections of juveniles from the other three areas also all gave length measurements greatly overlapping those of adults. There was no evidence that any of these snails had reached sexual maturity, since no eggs were ever found. It can be suggested, therefore, that in the case of *P. parkeri* full size is reached in the fall and that growth is not resumed in the early spring of the second season. A comparison of the length measurements of the late summer collections of juveniles from the four different areas (Graph 2 and Table 1) suggests that in the South Fishtail Bay area growth was considerably slower than in the other three areas. Snails collected here on August 20 to 23 were smaller than those collected at approximately the same date from the three other areas.

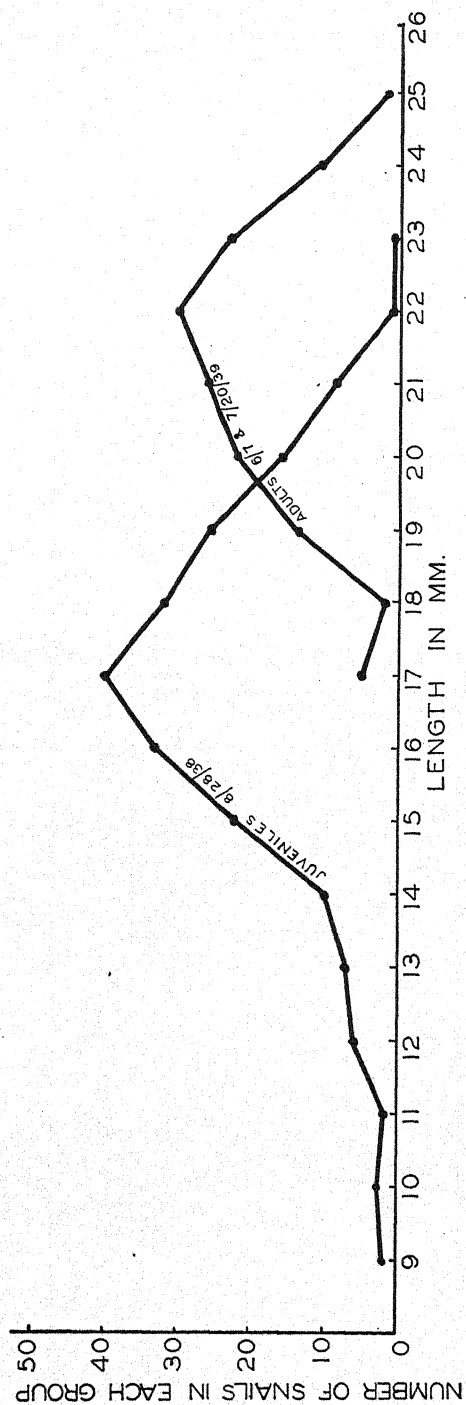
Growth between the time of the last fall collections and those made early the next June is shown in Graphs 1 and 3 for the snails from the Maple River and North Fishtail Bay areas and the same thing for the South Fishtail Bay area can be seen in Table 1. On the basis of these



GRAPH 1. Measurements of shell length of juveniles of *P. parkeri* from collections from the Maple River area on July 27, August 13, and September 3, 1938, compared with those of adults of the same generation collected in the early summer of 1939 (see Table 1).



GRAPH 2. A comparison of the length measurements of collections of juveniles of *P. parkeri* from the four areas made during the last half of the summer of 1938 (see Table 1).



GRAPH 3. Length measurements of juveniles of *P. parkeri* from a late summer collection in 1938 from the North Fishtail Bay area compared with those of adults from the same area collected early in the summer of 1939 (see Table 1).

data it seems reasonable to conclude that the full growth could easily have been attained in the fall before cold weather set in. It is also evident that there must have been a slowing up in the growth of the larger juveniles in all three areas, since the range of variation in the adults is less than in the juveniles of the previous summer. Here it can be suggested, as was done earlier for *S. emarginata angulata* (Cort et al, 1940a), that adult size may have been fixed by the beginning of cold weather, that the largest juveniles became the largest adults, and that the smallest juveniles remained small as adults. This suggests also the possibility that an important cause of variation in the adults, as in the juveniles, is the time of egg laying. According to this view, in general, those snails that hatched early in the summer became the largest adults and those from eggs laid late in the breeding season became the smallest adults.

The records of collections in Table 1 show that the adults of *P. parkeri* were scarcer on the beaches in the summer of 1939 than in 1938. It seems probable that a combination of factors led to this reduction in the numbers. In the Maple River area there was evidence that the juvenile snails were being killed in large numbers by exceptionally heavy larval trematode infections. The collections of the summer of 1938 were probably also a factor, especially in the South Fishtail Bay area, where we deliberately tried to collect out the snails because of danger of schistosome dermatitis. Other factors, probably adverse environmental changes, seem to have been operating in this reduction, especially in the Nutting Bay area, where the incidence of trematode infections was low in 1938 and where little collecting was done that summer.

Our collections on the beaches of Douglas Lake over a period of years had given the general impression that adults of *P. parkeri* are scarce after the middle of the summer and the collections of the summers of 1938 and 1939 confirmed this impression. Even in the earliest collections large numbers of empty shells were found, which showed from their color and condition that they belonged to adult snails that had recently died. Also, on every collecting trip we found shells which still retained the dead bodies of snails. In addition, it became increasingly hard to find adult snails in collections made after the middle of July. It is evident, therefore, that soon after the end of the breeding season, the adults of *P. parkeri* die and are almost entirely replaced by the new generation of juveniles by August.

The life span for *Physa parkeri*, therefore, is about 13 to 14 months, and extends over only one winter. Egg laying begins sometime in June and few if any eggs are laid after the middle of July. The juveniles grow very rapidly, the majority overlapping the adult size by the end of August, but do not attain sexual maturity until the next spring after pass-



ing the winter under the ice. After the breeding season the adults soon die, are scarce by the middle of July, and appear to be all gone soon after the first of August. As compared with *Stagnicola emarginata angulata*, the breeding season of *P. parkeri* appears to be somewhat earlier, the juveniles attain a relatively larger size by the end of the summer, and the adults die sooner in the summer.

LARVAL TREMATODE INFECTIONS IN JUVENILES AND ADULTS  
OF *Physa parkeri*

In the four areas on Douglas Lake *P. parkeri* harbors six species of larval trematodes (see Table 2), viz., a schistosome (*Cercaria physellae* Talbot, 1936), an echinostome (the cercaria of *Echinoparyphium recurvatum* (Linstow, 1873)), a strigeid (*C. physae* Cort and Brooks, 1928), a notocotylid (the cercaria of *Notocotylus urbanensis* (Cort, 1914)), and two plagiorchids (the cercaria of *Cercorchis medius* (Stunkard, 1916) and one belonging to the Reniferinae).

*C. physellae* is one of the dermatitis-producing schistosome cercariae of the region. It is found also in the small beach *Physa*, *P. magnalacustris*, and in the Douglas Lake region seems to be pretty well limited to these lake shore physae. It has been reported by Brackett (1940a) from other species of this genus in Wisconsin and is found there in a wide variety of habitats. In the Douglas Lake region it seems to be of little significance in the production of the "swimmer's itch" on the bathing beaches (Cort et al, 1940b). The adult of *C. physellae* is not known but the recent epidemiological studies of Brackett (1940b) in Wisconsin indicate that its definitive host is probably a duck.

The cercaria of *Echinoparyphium recurvatum* had a high incidence in our collections. Its adult has a wide range of hosts, both birds and mammals. It is common in the muskrats in the Douglas Lake region and its high incidence in the Maple River area (Table 2) was attributed to the presence on this beach of large numbers of these hosts. The cercaria of this species is also present in other species of *Physa* including those, such as *P. gyrina*, that live in ponds and swamps. It is also found in low incidence in *Stagnicola emarginata angulata*.

The strigeid, *C. physae*, is very similar to the cercaria of the heron strigeid, *Posthodiplostomum minimum* (Hunter, 1936 and 1937; Ferguson, 1936 and 1937). This cercaria is very widespread in the region and is found commonly in various species of *Physa*, including those from small ponds and swamps. It seems probable that its definitive host may be the great blue heron which is so common in the Douglas Lake region.

The notocotylid of our collections was shown experimentally by Herber (1939) to be the cercaria of *Notocotylus urbanensis*. The cercaria of this species is also widely distributed in the various species of *Physa* and is present in low incidence in *S. emarginata angulata*.

TABLE 2.—Larval trematode infections in *Physa parkeri* collected from four areas on Douglas Lake during the summers of 1938 and 1939

	Maple River area			South Fishtail Bay area			North Fishtail Bay area			Nutting Bay area	
	Juveniles		Adults	Juveniles		Adults	Juveniles		Adults	Adults	Juveniles
	7/11/38 7/27/38	7/27/38	9/3/38 6/15/39 6/24/39 7/4/39	7/27/38	7/27/38	6/18/38 7/7/38 7/21/38 7/25/38	8/22- 23/38	6/14/39 6/23/39 7/3/39	6/21/38		
Total collection	150	298	440	203	355	322	304	66	60	209	300
Negatives	25 (17)*	110 (38)	291 (66)	120 (59)	203 (83)	304 (94)	0	58 (88)	35 (59)	157 (75)	260 (87)
Immature (unidentified postives)	0	3	1	0	3	0	0	0	0	0	0
Multiple infections	4	2	16 (4)	34 (17)	0	1	1	0	1	1	0
<i>Cercaria physellae</i> —Total	24 (16)	4 (1)	15	2	34 (10)	1	1	5 (8)	18 (30)	5 (2)	0
Old	17	1	1	27	13	1	1	5	8	4	0
Mature	7	1	1	1	3	1	1	0	10	0	0
Young mature											
Immature											
<i>Cer. E. recurvatum</i> —Total	82 (55)	170 (57)	84 (19)	5 (17)	11 (3)	1	1	0	4 (7)	14 (7)	3 (1)
Mature	59	100	83	32	11	1	1	0	4	6	2
Immature	23	70	1	3	11	1	1	0	0	8	1
<i>C. physellae</i> —Total	4 (3)	12 (4)	37 (8)	6 (4)	2 (1)	15 (5)	1	0	0	23 (11)	10 (3)
Old											
Mature	1	10	34	4	2	1	1			17	7
Young mature										3	2
Immature										1	1
<i>Cer. N. urbanensis</i> —Total	17 (11)	2	3	4 (2)	12 (3)	3 (5)	3	3 (5)	4 (7)	3 (1)	27 (9)
Mature	17	1	7	3	12	3	1	3	4	3	14
Young mature											7
Immature											6
<i>Cer. C. medius</i> —Total	2 (1)	0	1	0	0	0	0	0	0	0	0
Mature	2	0	4 (1)	0	0	0	0	0	0	0	0
<i>Cer. Renferinae</i> —Total	0	0	0	3 (2)	0	0	0	0	0	8 (4)	0
Mature				2	0	0	0	0	0	8	
Immature				1	0	0	0	0	0	0	

\* Percentage incidence in collection.

The cercaria of *Cercorchis medius*, a turtle telorchiid (McMullen, 1934), was described from *Physa integra* and occurred in *P. parkeri* from only one of our areas.

The reniferid cercaria found in two of the collections is probably one of the two species for which the life cycles were described by Talbot (1933). As noted by this author it is almost impossible to make accurate identifications of these species from the cercaria alone. These cercariae are widely distributed and occur in several species of *Physa*. Their definitive hosts are garter snakes.

The incidence of infection of these six species of cercariae in *P. parkeri* is such that multiple infections would not be expected to be common except in the Maple River area (Cort et al, 1937). In fact in the whole series of examinations listed in Table 2 only nine double infections were found. *C. physellae* was found in three cases with the cercaria of *N. urbanensis*, once with the cercaria of *E. recurvatum*, once with *C. physae*, and once with an infection too young to be identified with certainty (probably *C. physae* since it had elongate daughter sporocysts). Also, the cercaria of *E. recurvatum* was found twice with that of *N. urbanensis* and once with the reniferid cercaria. An analysis of the chances of combinations of these species of cercariae occurring in double infections suggests that certain combinations must have been prevented by an immunity or antagonism. For example, there were no double infections of the cercaria of *E. recurvatum* and *C. physae*, although according to chance they should have occurred between 15 and 20 times in the collections from the Maple River area. Also *C. physellae* was found in only one double infection with the cercaria of *E. recurvatum* in the collections from this same area when according to chance this combination should have been found over 20 times.

The evidence indicates that juveniles of *P. parkeri* are infected with larval trematodes at all stages of development, that infections are carried over the winter in large numbers, and that adults also acquire infection after they pass the winter under the ice. The data on the infection of very young juveniles, which came from the collection of July 27 from the Maple River area are of special interest. From this collection the juveniles were individually measured at the same time they were examined for larval trematodes. Immature infections of the cercaria of *E. recurvatum* were found in four snails 3 and 4 mm in length and mature infections in nine individuals only 5 and 6 mm long. Also a mature infection of *C. physae* was present in a snail 6 mm in length, an old infection of *C. physellae* in one 9 mm, and a mature infection of *Notocotylus urbanensis* in one 10 mm. Other collections gave a small amount of similar evidence (Table 2). These findings, especially the presence in rather small snails of so many mature infections, which must have taken

a minimum of five to six weeks to develop, suggest that the miracidia penetrate the juveniles of this snail soon after they hatch from the eggs.

It is interesting to compare the infections with the cercaria of *E. recurvatum* in juveniles of different sizes collected from the Maple River area on June 27, 1938 (Table 3). In this analysis it appears that the

TABLE 3.—Infection with the cercaria of *E. recurvatum* in different sized juveniles of *P. parkeri* collected in the Maple River area on July 27, 1938

	Length in mm							
	3 and 4	5	6	7	8	9	10	11 to 17
Number in group .....	14	20	21	30	12	30	15	156
Negatives .....	9	11	11	18	6	14	5	36
Cer. <i>E. recurvatum</i> : .....								
Immature .....	4	4	4	6	4	5	3	40
Mature .....	0	5	4	4	1	10	5	71
Total .....	4	9	8	10	5	15	8	111

smaller snails tend to have a larger proportion of negative, and the proportion of immature to mature infections became progressively less in the larger snails. It seems evident, therefore, that the larger juveniles were older and consequently had a longer period of exposure to infection.

Evidence that infections of the juveniles are carried over the winter in the snails comes from the June collections of adults made in 1938 and 1939. In 1938 the ice broke up on Douglas Lake on April 12 and in 1939 on April 25 to 28. As noted earlier (Cort et al, 1940a) the temperatures of April and early May in this region are almost too low for development of larval trematodes and development would be slow until late in June. It seems almost certain, therefore, that at least the old and mature infections with larval trematodes that were found in adult snails in June (Table 2) had entered the previous season and had been carried over the winter.

There is, in addition, evidence of susceptibility of adults of *P. parkeri* to infection with larval trematodes. For example, in a collection of 114 adults from the Maple River area on July 11, 1938, there were 21 immature infections with the cercaria of *E. recurvatum*. Also, it can be noted from Table 2 that in the collections of adults from all the areas there was a considerable number of immature infections representing all but one of the species of larval trematodes. In addition, there were six infections too immature for identification. It, therefore, becomes evident that some adults of *P. parkeri* were being infected during their second season.

Some evidence concerning the time that infections with the different species of larval trematodes were acquired and their course in the snail hosts is found in Table 2. The low incidence made it impossible to get any evidence on these points for the two plagiogoriid species. Of the larval stages of *N. urbanensis* also little can be said, except that few im-

mature infections were found and the adult snails in general had higher incidences than the juveniles.

*C. physellae*. As brought out in an earlier paper (Cort et al, 1940a) the development period for schistosomes from the time the fully embryonated eggs are dropped into the water until the first cercariae escape from the infected snails would be, in the Douglas Lake region, about 5 to 6 weeks in the warmest part of the summer. A total of 76 infections of *C. physellae* was found in adult snails collected in June and July, 1938, from three areas. Of these, 43 infections were listed as old, 30 as mature, and 3 as young mature. In June the proportion of old infections was about 50 and in July about 60 per cent. It seems probable that all these old and mature infections had entered the snails the previous season, probably both late in the summer and early in the fall. The incidence of infection with *C. physellae* in the juveniles of *P. parkeri* was very low as compared with that of the adults. In the Maple River area there were only 4 infections in almost 300 juveniles collected July 27, 1 old, 2 mature, and 1 young mature. Even postulating the minimum possible time of development it is evident that these infections must have entered the snails when they were very small. Some infection of snails later this same summer is indicated by the presence of 15 mature and 1 young mature infection in the juveniles collected from this area on September 3. The adult snails collected in the early summer of 1939 in this area showed a greatly increased incidence of infection with *C. physellae* as compared with the juveniles of the previous summer from which they developed. When we consider that the season in 1939 was unusually late it seems almost certain that all these infections, except the five immature ones, had entered the juveniles the previous season. In the late August (1938) collections of juveniles from the South Fishtail Bay area only one infection of *C. physellae* was present and in that from the North Fishtail Bay area there were 5 (4 mature and 1 immature). Adults collected from the North Fishtail Bay area for the next summer showed a great increase in the incidence of *C. physellae*. While most of these infections probably came in the previous fall, the 6 immature infections which were found in the June 17–20, 1939, collection may have come in the same season.

Although the adult stage of *C. physellae* is not known, it seems almost certain, as indicated above, that it lives in a species of duck. These hosts must have been giving off the eggs of this schistosome on the beaches of Douglas Lake chiefly in the late summer and early fall to produce the large number of infections that were carried over the winter. Some slight infection of snails also occurred in the spring and first half of the summer as indicated by the infections found in juveniles. These relations and the characteristics of the life cycle of *P. parkeri* indicate



that the only time of the year that the cercariae of this schistosome would be escaping from its snail host in sufficient numbers to produce outbreaks of dermatitis in man would usually be in May, June, and early July before the swimming season got well under way. Even by the end of June the number of adult snails is considerably reduced by natural death and in a season such as 1938 a considerable proportion of the infections of *C. physellae* would have exhausted their supply of cercariae. The slight infection found in the juveniles would not be sufficient to produce enough cercariae to make them a factor in the dermatitis. Also the snails of this species are seldom numerous on the beaches. The fact is that only in one area where several people contracted the dermatitis early in the summer have we obtained any evidence of "swimmer's itch" produced by *C. physellae*. Therefore, this schistosome cercaria, as was suggested in an earlier paper (Cort et al, 1940b), can be almost entirely disregarded in relation to the problem of "swimmer's itch" in the Douglas Lake region.

*C. physae*. The data available in the literature (see van Haitsma, 1930, and 1931a and b) indicate that in strigeids the time of development from the dropping of eggs into the water until the first cercariae escape from the infected snails is about 8-10 weeks under favorable temperatures, the development in the snail requiring about 6 to 7 weeks. If we accept this time for the development of *C. physae* the data from our examinations (Table 2) suggest that infection of snails with this species takes place at various times during the "open" season. Immature infections in adult snails of the June and July collections suggest that they become infected in the late spring or early summer. Also, the finding of mature infections in juveniles in the collection of July 27, 1938, from the Maple River area indicates that the eggs of this species were dropped into the water in the spring and that the snails were infected in June. The presence of mature, young mature, and immature infections in juveniles collected from all four of our areas from August 23 to September 3 further shows that snails were infected during June, July, and perhaps early August. Mature infections in the adult snails were almost certainly derived from infections that were carried over the winter. It seems evident, therefore, that the definitive host of *C. physae* must visit the beaches and deposit the eggs in the water at various times in the spring, summer, and fall. The great blue heron, which was suggested above as a possible definitive host for *C. physae*, frequents the beaches throughout the summer, so that an adult strigeid in this host might well produce the kind of snail infection that was found for *C. physae*.

Another very interesting point about *C. physae* is that the incidence of infection is much higher in juveniles than in adults. In the three areas where we have late summer records for juveniles followed by examina-

tions of the adults next season, the incidence of *C. physae* in the latter has been greatly reduced (see Table 2). In the late summer collections of juveniles most of the infections with *C. physae* were already mature. What seems to us the most reasonable explanation for the lower incidence in adult snails is that infections with this cercariae that mature in the juvenile snails during their first summer become old during the fall, and disappear sometime during the winter, very probably by the death of the infected snails. This view is supported by the fact that only one old infection of *C. physae* was found in an adult snail. Infections of strigeids when they become old almost completely destroy the digestive glands of the infected snails and would naturally be injurious to growing juveniles. Evidence was also presented in an earlier publication (Cort et al, 1939) that infections of the gull strigeid, *Diplostomum flexicaudum*, cause the death of numbers of juvenile *S. emarginata angulata*.

*Cercaria of Echinoparyphium recurvatum.* The data on this species (Table 2) show infection of high incidence in both juveniles and adults. It is improbable that the juveniles of *P. parkeri* 5 to 6 mm in length that harbored mature infections of this larval trematode on July 27, 1938, on the beach in the Maple River area were more than a month old. It can be suggested, therefore, that the development of this species in the snail host under unusually favorable conditions can be completed in about a month.

As stated above we concluded that the large amount of infection in the snails of the Maple River area came from the muskrats. In the first collection of *P. parkeri* from this beach (July 11, 1938) sixty of the 114 adults were infected with this echinostome species (39 mature and 21 immature). On July 27, 36 adults were taken from the same beach. Twenty-two of these snails were infected with the echinostome but only two of the infections were immature. On this same date 298 juveniles, 3 to 17 mm in length, were also collected, of which 170 were infected with the cercaria of *E. recurvatum*. Seventy of these infections were immature. Thus large numbers of both juveniles and adults of *P. parkeri* were becoming infected with this echinostome early that season, although it is possible that the mature infections of the adults of the July 11 collection had in part at least been carried over the winter.

We noted in the examinations of the juveniles of the July 27 collection that the digestive glands of the snails were very severely damaged especially by the mature infections in which almost all the tissue was replaced by numbers of large rediae. The juveniles were very numerous on this beach on July 27 and our collection of about 300 snails reduced the numbers only slightly especially since we covered only a part of the beach. When we made our collection of September 3 the number of juveniles was much less than on the earlier date although they were

still fairly numerous and we were able to collect 440 in about three hours. The examination of this collection showed a very great reduction of the incidence of the cercaria of *E. recurvatum* from 57 per cent on July 27 to 19 per cent on September 3. This very striking reduction in incidence certainly suggests that the infections with this larval trematode were killing large numbers of these snails and were probably a significant factor in reducing their numbers. Also, the fact that 83 of the 84 infections of the cercaria of *E. recurvatum* found in the juveniles on September 3 were mature indicates that the infection of the snails was practically limited to the first part of the summer although the reason for this is not clear. When collections of adults of *P. parkeri* were made early the following summer, it was found that they were much scarcer than they had been the previous summer. As suggested earlier in this paper, this reduction in numbers was probably due to a large extent to the killing of great numbers of the juveniles by the echinostome infections. It seems probable also that most of the echinostome infections in these snails collected in 1939 had lived over the winter since only 2 immature infections were present.

While many workers have called attention to the injury to the snail hosts produced by larval trematode infections only a few reports present definite evidence indicating that they are an important lethal factor under natural conditions. Johnson (1920, pp. 363-364) suggested that infections with the cercaria of *Echinostoma revolutum* were an important cause of the death of the snails in a heavily infected pond where he made his collections. Wesenberg-Lund (1934, pp. 183-184) has also emphasized the importance of larval trematode infections in the reduction of snail populations. Our own data indicate particularly the death of juvenile snails brought about by infections of larval trematodes introduced so early that they are able to run their course and grow old before the maturity of the snail. The digestive glands of the snails are almost completely destroyed by old infections, especially of echinostomes, schistosomes, and strigeids, where the rediae and daughter sporocysts invade all the tissue. We are of the opinion that snails are unable to recover from such infections. They probably shorten the lives of adult snails, but seem to be especially important in their effects on snail populations by killing large numbers of juveniles before the breeding season.

#### SUMMARY

Investigations were made during the summers of 1938 and 1939 on the relations of larval trematode infections to the large beach *Physa*, *P. parkeri*, in four areas on Douglas Lake, Michigan. The life span of this snail species appears to be from about 13 to 14 months. The eggs are laid by the adults in the late spring and early summer, probably from

late May to early July. The period of embryonic development varies from 8 to 17 days according to the temperature and the newly hatched juveniles are about 1 mm in length. The juveniles grow rapidly on the beaches during the summer and by late August greatly overlap the adult size. They probably reach adult size in the fall. After passing the winter under the ice they are all sexually mature early the next summer. Soon after their breeding season, even before the end of June, they begin to die off rapidly and almost all are gone before the end of July. Therefore, collections of *P. parkeri* made at the end of the summer contain only large juveniles, while those of early June contain only sexually mature adults.

In the collections of both juveniles and adults of *P. parkeri* from the four study areas on Douglas Lake six species of larval trematodes were found, viz., a dermatitis-producing schistosome cercaria (*Cercaria physellae* Talbot, 1936), an echinostome (the cercaria of *Echinoparyphium recurvatum* (von Linstow, 1873)), a strigeid (*C. physae* Cort and Brooks, 1928), a notocotylid (the cercaria of *Notocotylus urbanensis* (Cort, 1914)), and two plagiurchiids (the cercaria of *Cercorchis medius* (Stunkard, 1914) and one belonging to the Reniferinae). Only 9 double infections were found involving 5 combinations of these six species of cercariae. It is suggested that there is some antagonism or immunity relation that prevents combinations in double infections of the echinostome with the schistosome and strigeid since each of these combinations would have otherwise occurred much more often.

The finding of adult infections in rather small juveniles suggested that they were infected when very young, probably soon after hatching. A large proportion of the infections found in the adult snails appears to have been carried over the winter, although the evidence is clear that some infections enter the adults early the second summer. In the case of the schistosome, *C. physellae*, it seems probable that most of the infections enter the juveniles in the late summer and early fall and are carried over the winter, because the incidence of the infections is much lower in the juveniles than in the adults.

Infections of the strigeid, *C. physae*, appear to enter the snails all through the open season on the lake, since immature infections were found in almost all collections, both of juveniles and adults. The great reduction of incidence of *C. physae* in the adult snails as compared with the juveniles suggests that infections that come to maturity in the juveniles kill them in considerable numbers.

The very high incidence of the cercaria of *E. recurvatum* in one area was attributed to the abundance of muskrats. This cercaria infected very young juveniles and came to maturity in numbers in those that were only partly grown. These infections apparently killed large num-

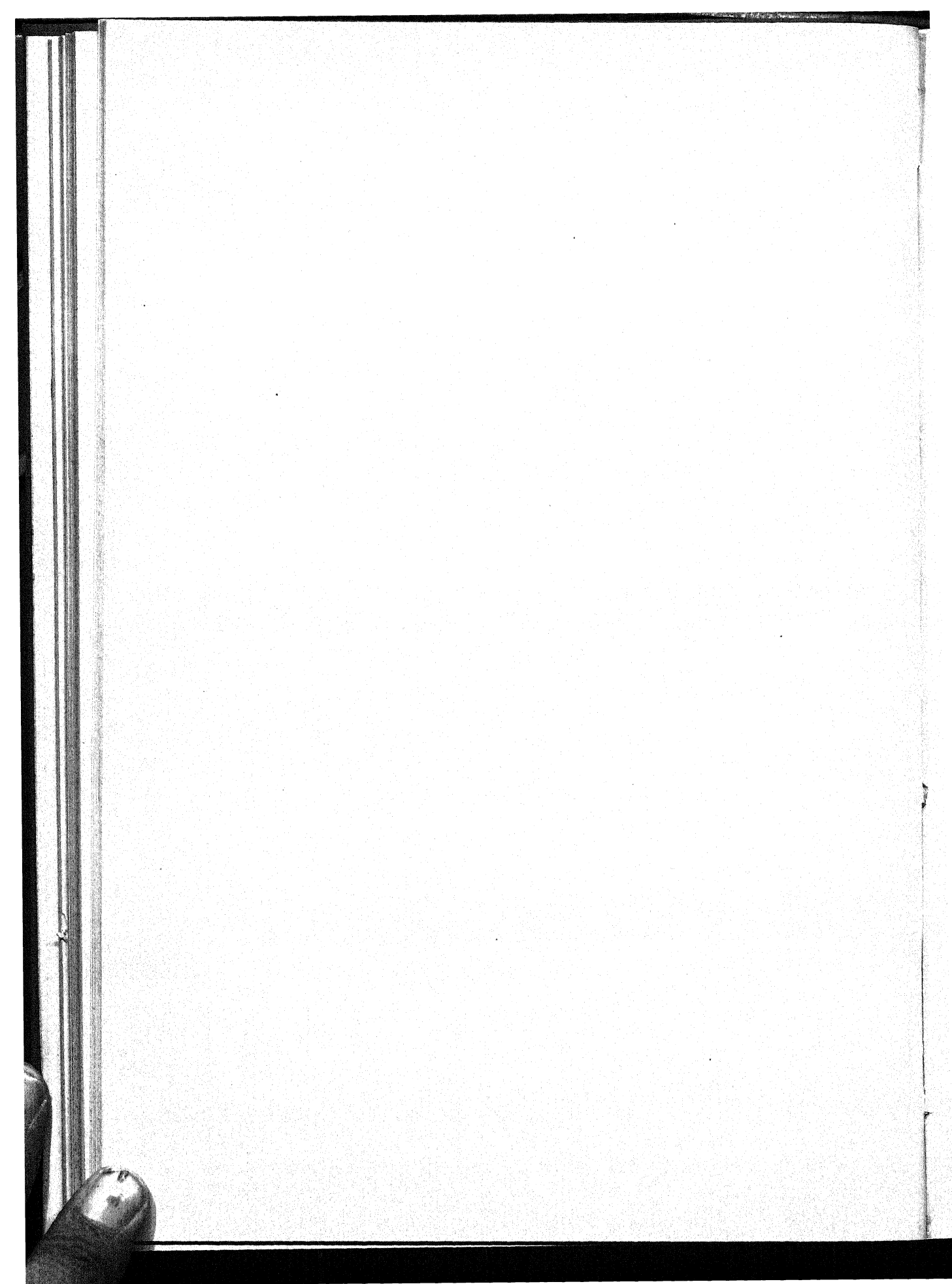
bers of the juvenile snails in this area. This and other species of larval trematodes, especially strigeids and schistosomes, as they grow older almost completely destroy the digestive glands of the infected snails. It seems probable, therefore, that snails never recover from such infections, and that wherever they mature in numbers in juveniles, they may be an important lethal factor which may affect adversely whole snail populations.

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# STUDIES ON PHENOTHIAZINE. IX. THE BILIARY EXCRETION AND ANTHELMINTIC ACTION OF THIONOL

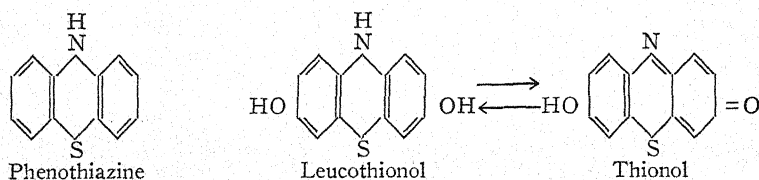
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Phenothiazine, described by Smith, Munger, and Siegler (1) in 1935, is one of the most effective, and certainly the most versatile, of the organic compounds developed by the U. S. Department of Agriculture. In addition to being a promising insecticide phenothiazine has been shown to be useful as a urinary antiseptic (2), and is now receiving considerable attention as an anthelmintic (3-7) for removal of parasites from farm animals, notably sheep.

In a previous paper (8), proof of the oxidation of phenothiazine to thionol after oral administration of phenothiazine to rabbits, rats, and man was given, and the excretion of thionol, leucothionol, and phenothiazine was discussed. At that time interest was limited largely to the urinary excretion of phenothiazine and its oxidation products because of the bactericidal properties (9) conferred upon the urine.

Recent observations have demonstrated the presence of phenothiazine, leucothionol, and traces of thionol in the feces, the oxidation product existing predominantly in the leuco form because of the reducing properties of the intestinal contents. In view of the bactericidal properties of thionol, the presence of phenothiazine, thionol, and leucothionol in the intestine has acquired a new interest following the reports of the anthelmintic action (3-7) of orally administered phenothiazine. The structural relationship of these compounds is shown in the following formulae:



If the conversion of phenothiazine to thionol and leucothionol, at least in part, takes place in the digestive tract, the presence of these three substances in the feces could be explained on the basis of incomplete absorption. If absorption from the digestive tract is complete, or if oxidation of phenothiazine takes place only after absorption, the presence of these

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compounds in the intestinal tract indicates an excretion into the intestine through the biliary tract.

#### EXCRETION IN RABBITS

To obtain information on the possibility of biliary excretion in this species two rabbits were used. One rabbit, weighing 3.7 kilograms, was given 3 grams of phenothiazine by stomach tube, and the other, weighing 3.5 kilograms, was given 3 grams of thionol. The rabbits were killed about six hours later and the bile removed from the gall bladders by means of a hypodermic syringe. Each bile sample was treated as follows: After diluting it with about 100 cc of distilled water and warming, concentrated hydrochloric acid was added until no further precipitation occurred. The sample was then filtered and extracted with ether in a separatory funnel. After evaporation of the ether the residue was dissolved in a small volume of 95 per cent alcohol, which yielded a red solution. The alcoholic solution was then diluted with water. Upon the addition of water the sample obtained from the rabbit given phenothiazine yielded a fine silky-appearing precipitate, indicative of unoxidized phenothiazine, which was removed by centrifuging. The supernatant dilute alcohol was now poured off. This reddish supernatant fluid gave an intensification of the red color upon the addition of either hydrogen peroxide or sodium hydroxide, thus indicating that both thionol and leucothionol were present in the bile. The sample of bile from the rabbit receiving thionol gave the same results, except that there was, of course, no silky precipitate indicative of phenothiazine. Examination of control bile gave negative results.

The positive results obtained demonstrate that the gastric administration of phenothiazine to these rabbits resulted in the biliary excretion of phenothiazine, thionol, and leucothionol, and that the gastric administration of thionol gave rise to the presence of thionol and leucothionol in the bile.

#### EXCRETION IN DOGS

Biliary excretion of thionol in this species was demonstrated with three animals. Three dogs weighing 14.8, 12.5, and 10.8 kilograms were given 1.75, 1.75, and 1.5 grams of phenothiazine respectively in gelatin capsules. Six hours later when these dogs were operated upon in the laboratory of experimental surgery, bile samples were obtained with sterile hypodermic syringes. The bile samples were examined as outlined for the experiment on rabbits. Each of the three samples of bile gave positive evidence for the presence of thionol, leucothionol and traces of phenothiazine.

#### EXCRETION IN MAN

While treating patients having urinary tract infections with orally

administered phenothiazine, Dr. A. B. Stockton of the Department of Medicine encountered a patient with a biliary fistula. This fortuitous circumstance permitted the collection of a sample of bile before and after the administration of phenothiazine. The human bile thus obtained was examined as outlined for rabbits. The control sample of bile, obtained prior to administration of phenothiazine, gave negative tests, but the sample following use of the drug gave positive evidence for the presence of phenothiazine, thionol and leucothionol.

In each instance where a red dye was removed from the bile, the product displayed the reactions typical of thionol. It could be easily extracted from aqueous solution by either ether or chloroform. In aqueous solution it could be easily reduced to the leuco form by stannous chloride or sodium hydrosulfite, and reoxidized with hydrogen peroxide.

These evidences for the biliary excretion of phenothiazine, thionol, and leucothionol in rabbits, dogs, and man suggest an explanation for the presence of the substances in the intestinal tract, over and above any unabsorbed phenothiazine, or thionol and leucothionol formed from phenothiazine in situ.

When considered in the light of the earlier reports (2) on the bactericidal properties of phenothiazine, it is suggested that the therapeutic value of orally administered phenothiazine be tested in cases of cholecystitis where bacterial infection is known or suspected of being present. Parasitocidal effects might be expected in the intestine, and indeed an anthelmintic action has been reported by parasitologists.

#### ANTHELMINTIC ACTION

Phenothiazine is remarkable in that, following gastric or oral administration, it is rapidly absorbed despite its low water solubility. A part of the phenothiazine administered is oxidized to the reversible oxidation-reduction system thionol-leucothionol. At present it is not known whether this oxidation takes place in the digestive tract or in the tissues following absorption of phenothiazine. In any event there is excreted in the urine (8) and in the bile phenothiazine, thionol and leucothionol.

In the urine, at least, there is also leucothionol in some conjugated form from which it is liberated easily by acid hydrolysis. The exact manner of conjugation is not understood at present, nor is it known how the phenothiazine is kept in solution in bile and urine. When precipitated from these media by the addition of hydrochloric acid, phenothiazine cannot be brought back into solution by neutralization with sodium hydroxide. Even if phenothiazine is completely absorbed, its presence together with thionol and leucothionol in the bile would explain the presence of all three compounds in the intestinal tract.

The conversion of phenothiazine to thionol takes place readily in the



presence of air and moisture and, in the presence of appropriate reducing agents, variable amounts of thionol are reduced to leucothionol. If shaken with water to form a saturated solution, phenothiazine will, in the course of two or three days, be converted to thionol and confer a red color to the solution. Chemically, thionol is closely related to the other two thiazine dyes, Lauth's violet and methylene blue, and like them is a reversible oxidation-reduction system. This system has been studied potentiometrically by DeEds and Eddy (10) and found to differ from the other two thiazines by being much more easily reduced. Its potential places it much closer to the oxygen electrode than either Lauth's violet or methylene blue. At pH 7 the  $E_o'$  for thionol is 0.158 volts, or about midway between 1-naphthol-2-sulfonate-indophenol and 2, 6-dichlorophenol-endo-ortho-cresol.

This ready conversion of phenothiazine to thionol and the position of the latter on the oxidation-reduction scale may have an important bearing on the mechanism of action as a bactericidal agent, insecticide, or as an anthelmintic. All living things have one characteristic in common, namely, respiratory processes, whether they be aerobic or anaerobic. The presence of a strongly positive oxidation-reduction system in adequate amount could conceivably interfere with the fundamental respiratory mechanisms. Thionol-leucothionol occupies a position on the oxidation-reduction scale much more positive than the natural oxygen carriers, cytochrome, pyocyanine, flavoprotein, ascorbic acid, flavine phosphate, and lactoflavine; and more positive than such reversible enzyme systems as succinic-fumaric, lactate-pyruvate, and xanthine-uric acid.

The remainder of this paper presents the evidence obtained from experiments in vitro to show that thionol has a definite effect on hog ascaris (*Ascaris lumbricoides*) whereas phenothiazine has no action as compared with an appropriate control. As stated above, the administration of phenothiazine leads to the biliary excretion of phenothiazine, thionol, and leucothionol which subsequently pass into the intestinal tract. Thionol is therefore present in the intestine and available for an anthelmintic action if such exists.

Hog ascaris and a sample of hog bile were obtained from a local slaughter house, taken to the laboratory as rapidly as possible, and kept in an incubator at 37° C until ready for use. A series of five conical-shaped urine sedimentation glasses were placed in a water bath maintained at a temperature of 37°-39° C. The basic medium employed in each vessel was 0.9 per cent sodium chloride. The first control medium was prepared by adding 10 cc of chloroform to 100 cc of saline solution, boiling to expel the chloroform, and making up to 100 cc with distilled water. This was done so that the saline control would contain traces of chloroform, if any, comparable to the amounts remaining in the vessels

containing the saturated phenothiazine and thionol solutions. The second control medium was the same as the first, except that 5 cc of hog bile were added. A saturated phenothiazine solution was prepared by adding 10 cc of a chloroform solution of phenothiazine to 100 cc of saline solution, boiling to expel the chloroform, filtering while hot, and making up to 100 cc with distilled water. A saturated thionol solution was made in a similar manner. The fifth vessel contained a saturated thionol solution prepared in the same way, but with the addition of 5 cc of hog bile. When the temperature of the 100 cc medium in each vessel reached equilibrium with that of the water bath, two active worms were placed in each vessel. This experiment was performed five times, the only variable being the amount of bile added to the fifth vessel. The periods of observation varied from two to six hours. The results of a typical experiment are given in Table 1.

The results obtained were the same and surprisingly clear-cut in all the tests made. The worms in both controls and in the phenothiazine solution remained active throughout the periods of observation. The worms placed in the thionol solution exhibited increased activity, and in some instances attempted to crawl up the sides of the vessel. This increased activity, indicative of irritation, was followed by depression and complete inactivity. As the experiment progressed there was, however, some return of activity which tended to increase as the intensity of color of the thionol solution decreased, a phenomenon to be discussed presently. The worms placed in the thionol solution to which 5 cc of bile had been added became depressed and inactive very early, showing little if any evidence of a preliminary stimulation or irritation. In other words, the presence of bile shortened or eliminated the first stage of action and facilitated development of the depressant action. However, the tendency to regain activity was present as in the thionol solution, but occurred earlier, and the disappearance of the characteristic red color of thionol took place sooner.

The disappearance of color in the thionol and thionol plus bile solutions may be considered in conjunction with the observed resumption of activity by the worms. In the case of thionol alone, the worms became slightly red in color, while the medium was losing color. The loss in color of the medium was undoubtedly due, in part, to a taking up of thionol by the worms, but was due in large measure to a reduction of thionol to leucothionol. Proof of the existence of leucothionol in the medium was obtained by adding hydrogen peroxide to a few cc of the bleached medium and obtaining an intensification of color. As stated above, the worms placed in a thionol solution containing 5 cc of bile showed an earlier onset of depression, and an earlier return of activity, which coincided with a more rapid disappearance of the thionol color.

TABLE 1.—Comparative effects of phenothiazine and thionol on *Ascaris lumbricoides*

Time	Control 1 0.9 per cent sodium chloride	Control 2 0.9 per cent sodium chloride plus chloroform (see text)	0.9 per cent sodium chloride saturated with phenothiazine	0.9 per cent sodium chloride plus 0.01 per cent thionol	0.9 per cent sodium chloride plus 0.01 per cent thionol plus 5 cc bile
10:46	Active	Active	Active	Activity greater than controls	Least active
10:51	"	"	Few escape efforts	Many escape efforts	Activity increasing
11:00	"	"	Active	Escape efforts	Active but less so than controls
11:15	"	"	"	Little activity	Least active
11:30	"	"	"	"	Inactive
11:43	"	"	"	"	"
12:00	"	"	"	"	"
12:30	"	"	"	"	"
12:50	"	"	"	Very little activity	Slight activity
1:10	"	"	"	No activity	"

Since the control containing bile had no depressant action on the worms, the effect of bile in the presence of thionol must have been due to some factor such as the lowering of surface tension which might facilitate penetration of the dye into the tissues. Since bile itself causes some reduction of thionol to leucothionol, thereby lowering the concentration of effective thionol, and since the worms tend to recover their activity when the thionol is reduced, the reason for the more rapid recovery of the worms in thionol plus bile is evident.

Since bile facilitates the depressing action of thionol, but later hastens the return of activity, smaller amounts of bile might preserve the synergistic effect on the depressant action, but not convert sufficient thionol to leucothionol to facilitate recovery. Therefore additional experiments employing thionol plus bile were performed using 1 cc, 0.5 cc, 0.1 cc, and 0.05 cc of bile. In the vessel containing thionol plus 0.05 cc of bile the worms behaved about the same as in a medium of thionol alone. The other concentrations definitely hastened the depressant action of thionol, but did not cause sufficient reduction to leucothionol to appreciably shorten the period of depression. In other words, in the presence of a given amount of thionol there was an optimum concentration of bile which produced depression of the worms without shortening the period of recovery.

These *in vitro* results leave no doubt that, under the conditions of the experiment, thionol and not phenothiazine depresses the activity of hog ascaris, and that the effect may be augmented by an appropriate concentration of bile. The transfer of information obtained *in vitro* on one species of parasite to any explanation of anthelmintic action, in general, is recognized as lacking a certain validity. However, it must be granted that *in vitro* experiments do permit a control of conditions, and an examination of factors difficult to study in the intact animal. Finally, much of the value of such data depends upon the interpretation.

The evidence at hand shows that thionol, as well as leucothionol and phenothiazine, are present in the intestinal tract following administration of phenothiazine due to excretion in the bile. The depressant action of thionol plus bile observed *in vitro* was of limited duration, the time being determined by the time required to reduce the thionol to leucothionol. If conditions would permit the reoxidation of leucothionol to thionol, or if the concentration of thionol were maintained by the addition of a fresh supply the depressant action would likely persist. These theoretically more favorable conditions exist in the intact animal. The administration of phenothiazine may be repeated, and even with a single dose there tends to be a prolongation of action as the biliary excretion of thionol rises to a peak and then subsides relatively slowly. Meanwhile intestinal peristalsis would tend to remove the parasites before recovery is complete. The

evidence presented is, at least, highly suggestive that the anthelmintic action of phenothiazine is due to the thionol excreted into the intestine by way of the biliary tract and that bile facilitates this action.

#### MECHANISM OF ACTION

It is both desirable and fortunate that this effective anthelmintic exhibits little or no toxicity to the host. Although the argument may be regarded as highly speculative it is appropriate to make a suggestion as to the mechanism responsible for an effective toxic action toward bacteria, insects, and intestinal parasites, whilst mammals, dependent upon the hemoglobin system for oxygen transport, show few if any toxic symptoms. A detailed discussion of a probable mechanism would lead far afield into the complicated subject of biological oxidation, quite outside the scope of the present paper, but a suggestive hypothesis may be briefly stated.

At a pH of 7 the potential of the hemoglobin-methemoglobin system is +0.152 volts. At the same pH the potential of the thionol-leucothionol system is +0.158 volts. This slightly more positive potential of the thionol-leucothionol system is not likely to cause an appreciable shift of the hemoglobin-methemoglobin system toward methemoglobin and interfere with the oxygen transport mechanism characteristic of hemoglobin-oxyhemoglobin. In other biological systems, however, where oxygen transport is dependent upon such natural oxygen carriers as cytochrome, pyocyanine, flavoprotein, ascorbic acid, flavine phosphate, and lactoflavine, a different situation prevails. These systems are all much more negative in potential, could easily reduce thionol to leucothionol, and in turn be oxidized. Especially under conditions where a fresh supply of thionol is being supplied by oxidation of phenothiazine, as in the use of phenothiazine for anthelmintic purposes, for treatment of cystitis of bacterial origin, and in the slow but persistent oxidation of phenothiazine used as an insecticidal spray, it is conceivable that the natural oxygen carriers might be kept in an oxidized state, unable to function, and thus cause the depression and ultimate death of the organism.

#### SUMMARY

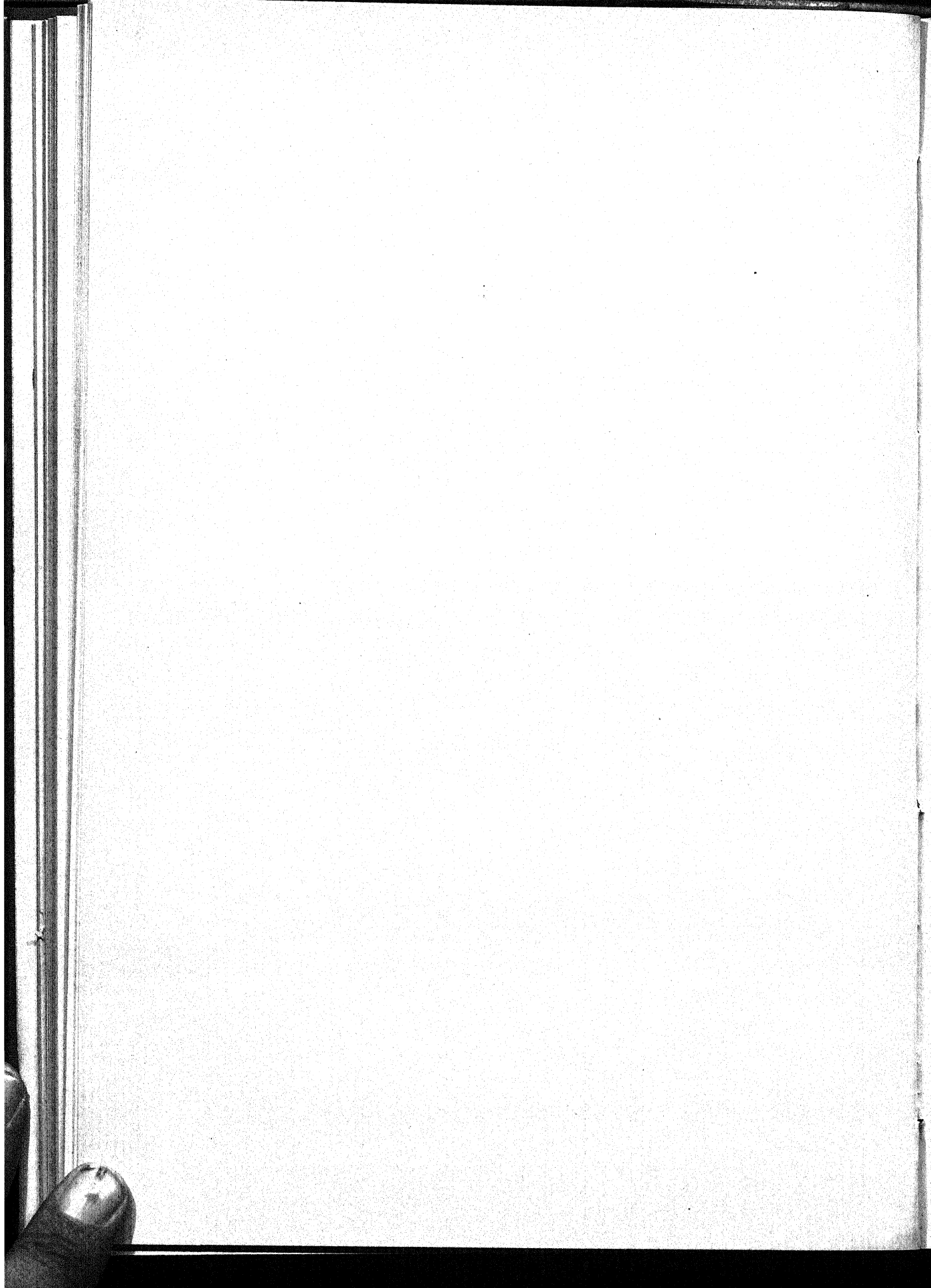
1. Gastric or oral administration of phenothiazine has been shown in one man, two rabbits, and three dogs to result in the biliary excretion of phenothiazine, thionol, and leucothionol.
2. In vitro phenothiazine had no demonstrable action on hog ascaris.
3. Thionol first caused stimulation of hog ascaris, followed by depression which persisted as long as an adequate concentration of thionol was present.
4. Bile facilitated the development of the depressant action of thionol on hog ascaris.



5. An hypothesis for the mechanism of thionol action has been briefly outlined.

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DIPLORCHIS SCAPHIOPI, A NEW POLYSTOMATID MONO-  
GENEAN FLUKE FROM THE SPADEFOOT TOAD\*

L. ORMAND RODGERS

Twenty of 62 spadefoot toads, *Scaphiopus bombifrons* Cope, collected near Stillwater, Oklahoma, in April and June 1940, were found to harbor 45 specimens of an apparently new ovoviviparous polystomatid fluke of the genus *Diplorchis* Ozaki, 1931. The parasites were found in the urinary bladder, 5 being the maximum number from a single host. The worms obtained in April were all mature and contained fully developed embryos. Indeed, eggs with delicate external membranes were deposited in large numbers in water in which the worms were collected. These quickly hatched, producing active, externally ciliated, tetraaculate larvae. The eyespots and moving cilia of larvae in utero could be seen in adults under low magnification. Some of the worms collected in June were small and immature; in these the reproductive organs were only partially differentiated and eyespots, in various stages of disintegration, were still discernible. Mature flukes taken in June contained and deposited fewer embryonated eggs than those of April.

The trematode was studied both alive and preserved. Fixatives used were Bouin's and acetic-sublimate, the stain borax-carmin. Measurements were taken from preserved specimens which had not been subjected to pressure at the time of killing. Mechanically flattened animals, however, were much more useful for the study of anatomical details.

*Diplorchis scaphiopi* n. sp.

(Figs. 1-5)

*Description:* Measurements (length  $\times$  width, unless otherwise stated) from 14-17 preserved and mounted adult specimens of the April collection. Total length 3.39 (2.15-4.35) mm. Body proper 2.45 (1.49-3.12)  $\times$  1.31 (0.78-1.78) mm. Haptor 763 (535-959)  $\times$  943 (666-1233)  $\mu$ . Oral sucker 173 (140-219)  $\times$  228 (180-280)  $\mu$ . Oral aperture 95 (73-126)  $\mu$  wide. Pharynx 163 (112-213)  $\times$  110 (58-166)  $\mu$ . Cirrus pouch 46 (40-52)  $\mu$  wide. Ovary 209 (133-466)  $\times$  125 (60-286)  $\mu$ . Testes too vaguely outlined and difficult to locate in adult unflattened specimens for measurement. Macrohooks 265 (179-406)  $\mu$  long. Microhooks 23 (18-28.5)  $\mu$  long. Acetabula 245 (159-326)  $\mu$  in diameter.

Body proper lanceolate, somewhat pointed at anterior end. Haptor subhexagonal and provided ventrally with 6 prominent acetabula, 16 microhooks, and 2 macrohooks. Positions of vulvae represented by paired marginal prominences at about one-third length of body proper from front end. Integument covered everywhere with a pattern of minute, contiguous, polygonal areas readily visible only under oil immersion objective; skin also densely beset with minute short papillae. In subadult animals the 2 tandem pairs of black-pigmented embryonic

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eyespots may still be seen, located dorsal to the front end of the pharynx, the front spots being slightly the closer together. Eyespots were not observed in larger specimens. Microhooks located, 1 in each acetabulum, 6 in a transverse row between the anterior acetabula, and 4 in a row near posterior margin between the back suckers. Macrohooks compressed, close together, with their broad, longitudinally striated, bases near center of haptor and their ventrally directed points farther back.

Oral sucker terminal, mouth ventrosubterminal. Pharynx pyriform, small end forward. Ceca clearly demonstrable only in living specimens. In young animals the ceca are very broad, have forward extensions lateral to the pharynx, are confluent in the haptor, and are provided with numerous short lateral and longer medial diverticula. In older animals the latter, on opposite sides of the body, become connected by a dozen or more irregularly arranged transverse commissures, which in turn may themselves be connected by longitudinal anastomoses. Many of the parasites, when first collected, had the enteron filled with a brownish fluid presumed to be partially digested blood. Numerous pigmented cells, clearly recognized only in living specimens, occur associated with (in or on) the walls of the ceca. These cells are circular to ellipsoidal in outline, flattened parallel to the intestinal wall, and rather uniformly distributed over the enteron although widely separated from each other. In each cell the hyaline nucleus and numerous discrete jet-black granules in the cytosome were clearly visible. After preservation these chromatophores cease to "stand out" individually and the pigmentation soon fades.

Paired urinary vesicles spheroidal, dorsosubmarginal between levels of pharynx and vulvae, with dorsal pores. In live worms the bladders were seen to pulsate frequently and alternately.

Testes symmetrically paired, large and subequatorial in young specimens, relatively smaller and farther forward in older ones. Vasa efferentia extend medio-subanteriad, uniting to form long sinuous forward-running vas deferens. Cirrus pouch located ventrosubmediad behind pharynx, armed with 8 hair-like setae.

Ovary ovoidal, longitudinally elongate, located to one side (right or left in equal frequency) of median line at level between vulvae and testes. Oviduct issues directly posterior from back end of ovary; it soon curves sharply ventroantero-mediad; next it gives off the genitointestinal canal which runs laterad ventral to oviduct to join ventral side of intestinal ramus ipsilateral with ovary; next it gives off the submedian common forward-reaching vitelline duct or vitelline reservoir; thence it curves abruptly posteriad and enters Mehlis' gland. The latter, relatively larger and more diffuse in young than in older worms, occupies more or less completely the interstice between ovary and testes. Uterus makes a principal posteriad loop reaching to or into haptor; descending limb slightly sinuous and largely confined to median plane; ascending limb, in adults, thrown into numerous transverse loops that extend across almost entire width of body, dorsal to descending limb. Uterus and cirrus pouch connect with a narrow common genital atrium which opens to exterior through a submedian pore ventral to pharynx.

Vitellaria a submarginal pair of small compact bodies at level between vulvae and ovary. Paired vitelline ducts run posteromedial to join short submedian common duct extending back to oviduct. Anterior end of common vitelline duct sometimes enlarged as a yolk reservoir. Vaginae extend medioposteriad from vulvae along medial surfaces of vitellaria to join proximal ends of paired vitelline ducts.

Along length of uterus one observes eggs, surrounded by delicate membranes, in all stages of development from unicellular zygote to ready-to-hatch larva. Most of ascending limb is occupied by the latter to the number of 2 or 3 dozen.

*Free-swimming larva:* Measurements from several preserved specimens. Body 403 (333-446)  $\times$  174 (139-213)  $\mu$ . Oral sucker 69 (61-79)  $\times$  85 (67-97)  $\mu$ ; mouth 32 (22-40)  $\mu$  wide. Pharynx 47 (34-63)  $\times$  34 (28-42)  $\mu$ ; prepharynx 26 (18-39)  $\mu$  long. Haptor 113 (100-153)  $\times$  146 (126-179)  $\mu$ .

Body ellipsoidal, a little more pointed anteriorly and with haptor well defined but not sharply set off. Microhooks similar in shape, size and number to those of adult. Macrohooks represented by partially developed feebly chitinized, rod-like

bodies. Large ciliated epithelial cells in undetermined number and arrangement more or less cover body proper and dorsal surface of haptor. Cerebral ganglia and interconnecting supraprepharyngeal commissure clearly observed. Two pairs of large heavily pigmented eyespots, the posterior ones being a little the larger, occur in direct tandem arrangement associated with antero- and posterodorsal surfaces of ganglia. In living specimens eyespots are deep-black in transmitted light, milky white and opalescent in reflected light.

*Host*: *Scaphiopus bombifrons* Cope, 1863.

*Habitat*: Urinary bladder.

*Locality*: Stillwater, Oklahoma.

*Cotype specimens*: Several in toto mounts in U. S. Nat. Mus. Helm. Coll.

The genus *Diplorchis* Ozaki, 1931, contains three previously known species, namely, *D. ranae* Ozaki, 1931, from *Rana rugosa* in Japan, *D. nigromaculatus* Lee, 1936, from *Rana nigromaculata* in China, and *D. americana* Rodgers and Kuntz, 1940, from *Scaphiopus couchii* in Oklahoma. The writer cannot offer a comparison of the new species with *D. nigromaculatus* because prolonged and repeated efforts to obtain the description (Lee, 1936) of the latter were in vain.

*Diplorchis scaphiopi* differs from *D. ranae* in having (1) integumentary papillae; (2) the microhooks more uniform in shape and size, the 2 hindmost pairs forming a transverse row; (3) compact vitellaria; (4) 8 instead of 10 setae in the cirrus pouch; (5) ovoviviparity; (6) a scaphiopodid instead of a ranid host; (7) a New World distribution. The new species differs from *D. americana* in being (1) less than half as large with regard to most of the principal measurements—the larva is also smaller, and in having (2) integumentary papillae, (3) macrohooks—this difference true of both adult and larva, (4) 8 instead of 6 setae in the cirrus pouch, and (5) the eyespots of the larva heavily pigmented.

*Diplorchis* belongs to the family POLYSTOMATIDAE Gamble, 1896, an important review of which is that of Price (1939).

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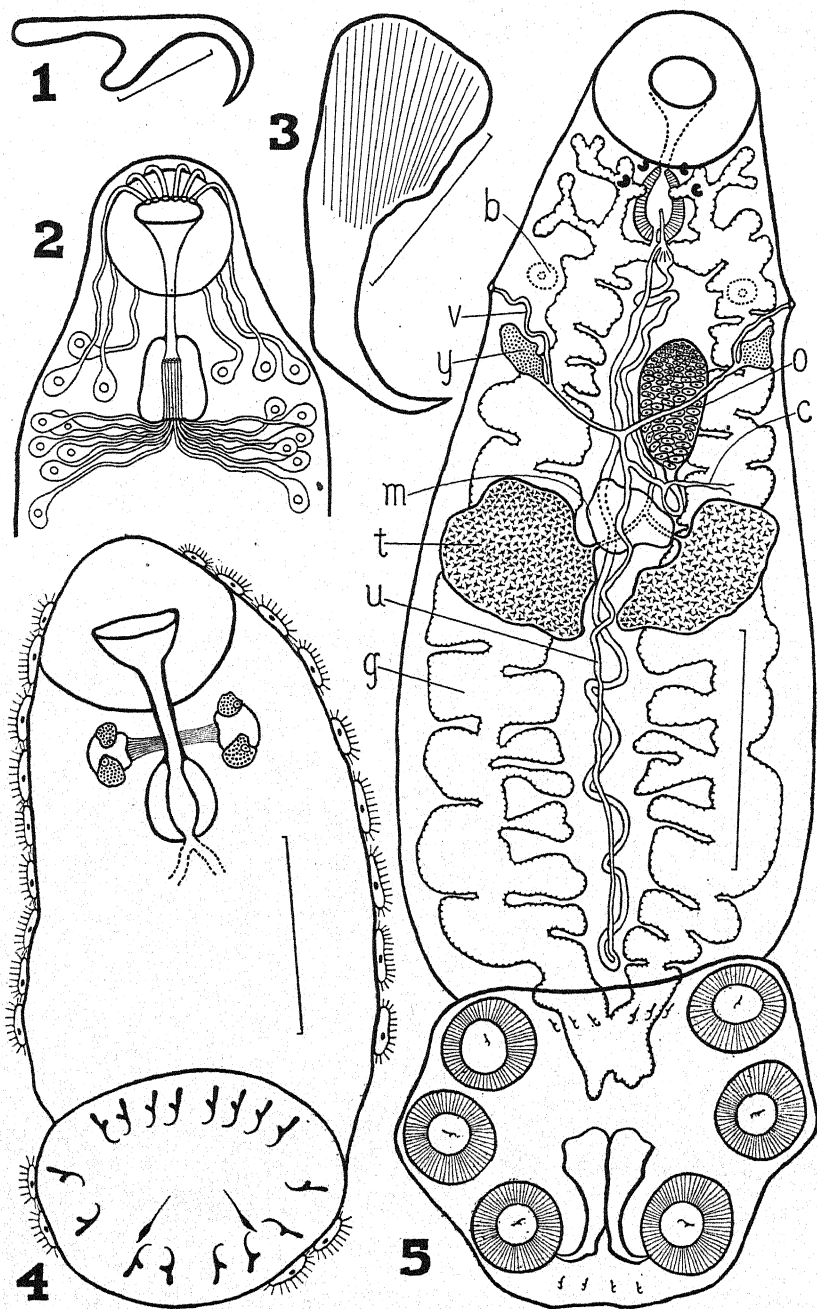
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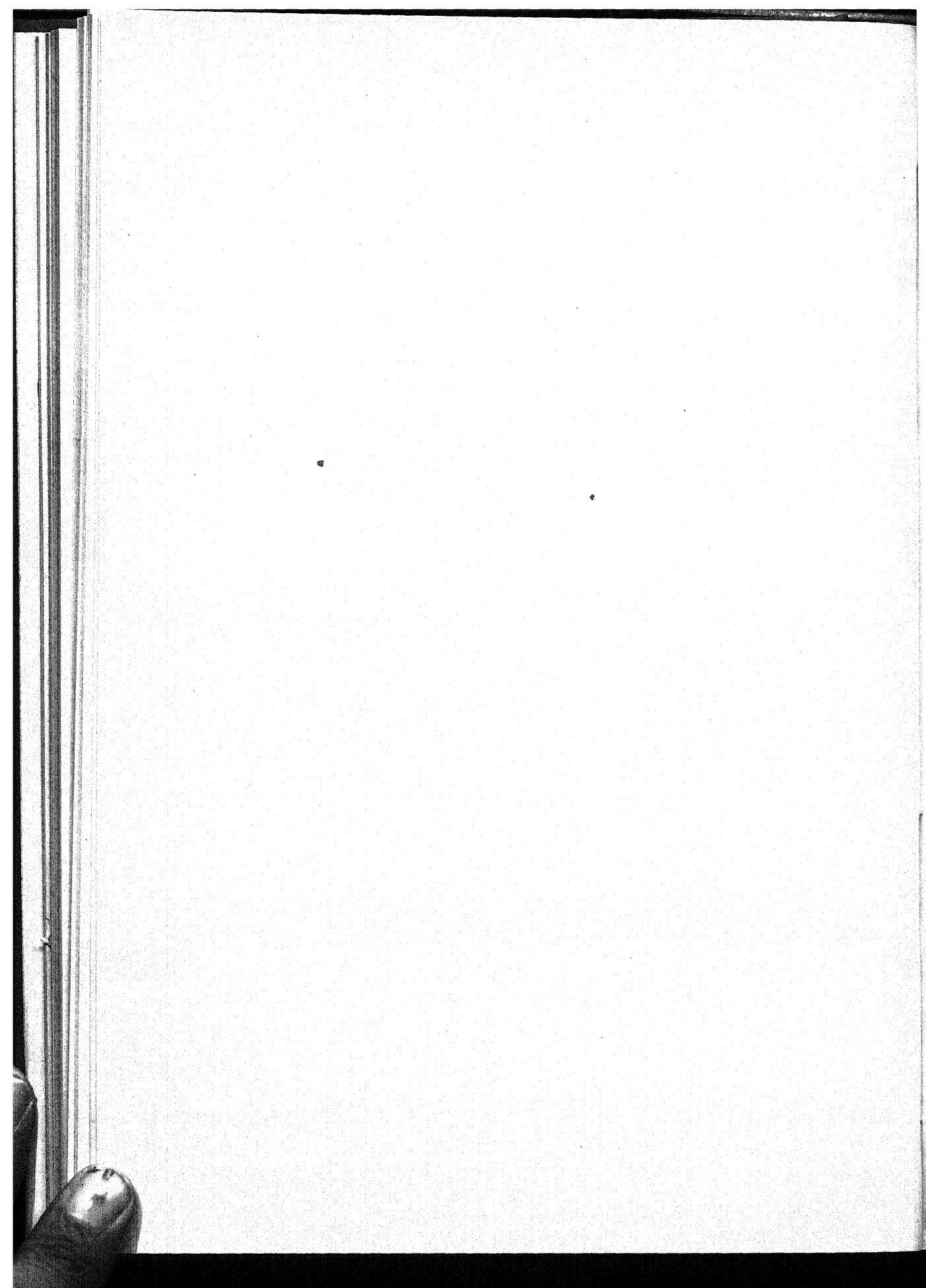
All figures concern *Diplorchis scaphiopi* n. sp. Fig. 2 is a freehand study; the others were drawn with the aid of a camera lucida with details added free-handedly.

## ABBREVIATIONS

b—excretory bladder	t—testis
c—genitointestinal canal	u—uterus
g—enteron	v—vagina
m—Mehlis' gland	y—vitellarium
o—ovary	

- FIG. 1. Small haptoral hook from an adult. Scale, 12.5  $\mu$ .  
FIG. 2. Unicellular glands about mouth and pharynx. The exact number of cells in either group was not determined.  
FIG. 3. Macrohook. Scale, 100  $\mu$ .  
FIG. 4. Free-swimming larva, ventral view. Scale, 100  $\mu$ .  
FIG. 5. Subadult, ventral view. The uterus and enteron have not reached their maximum complexity. The ovary and testes are relatively larger and more posteriad than in older animals. Scale, 500  $\mu$ .





STUDIES ON MONOGENETIC TREMATODES. IV.  
*ANCHORADISCUS*, A NEW DACTYLOGYRID  
GENUS FROM THE BLUEGILL AND THE  
STUMP-KNOCKER SUNFISH<sup>1</sup>

JOHN D. MIZELLE

The present author has been opposed to the creation of new genera of TETRAONCHINAE on the basis of characters of questionable value. In 1938 he and Hughes reduced nine North American genera to three, because of character differences considered insufficient for separation.

During the examination of rather extensive material collected from Florida fishes and kindly sent to the author by Dr. R. V. Bangham (College of Wooster, Wooster, Ohio), a type of TETRAONCHINAE, one species of which was described by Summers (1937) as *Actinocleidus triangularis*, has been found to differ enough in the nature of the haptoral armament to warrant generic distinction. The writer feels that the characters distinguishing this proposed genus are of sufficient magnitude to stand the test in a revision of North American TETRAONCHINAE which is to come at a later date. The present author believes that the described forms of North American TETRAONCHINAE are inadequate in number for presentation of a good picture of present evolutionary radiation, expressed in morphological detail, and that until the picture has been rendered more complete, a comprehensive taxonomic revision would be untimely.

*Anchoradiscus* n. g.

*Diagnosis:* TETRAONCHINAE with anchor bases situated parallel to, and developed so as to extend through the greater portion of a frontal plane of, the discoidal haptor. Anchor shafts vestigial or wanting; anchor points (recurved) directed laterally. Haptoral bars two, attached to the ventral (lateral) surfaces of the anchor bases and articulated with each other in midportions. Each of the fourteen hooks situated on the haptoral margin and differentiated into a base, a solid slender shaft, a sickle-shaped termination, and an opposable piece. Copulatory complex consisting of a cirrus and accessory piece; vagina sinistral. Eye spots four, posterior pair larger. Parasitic on the gills of fresh-water fishes.

*Type species:* *Anchoradiscus anchoradiscus* n. sp.

Received for publication, July 6, 1940.

<sup>1</sup> Contribution No. 76 from the Zoological Laboratory, Oklahoma Agricultural and Mechanical College, Stillwater.

Grateful acknowledgment is hereby made to Dr. W. A. Summers of Tulane University, for loan of figures and other information concerning *Anchoradiscus triangularis* (Summers, 1937) and also to Dr. E. W. Price of the Zoological Division of the Bureau of Animal Industry (U. S. D. A.), for checking the validity of the proposed generic name, *Anchoradiscus*.

*Anchoradiscus anchoradiscus* n. sp.

(Figs. A-D)

**Description:** Medium-sized dactylogyrids with a smooth cuticula; cephalic lobes usually well defined. Length 0.692 mm (0.456-0.945 mm), greatest width 0.187 mm (0.104-0.323 mm). Eye spots four, members of the posterior pair larger and closer together than those of the anterior pair. Pharynx circular or sub-circular in outline and may be broader than long or longer than broad; greatest transverse diameter 0.049 mm (0.029-0.065 mm).

Haptor discoidal in shape and of a diameter greater than the body width; greatest transverse diameter 0.313 mm (0.185-0.484 mm). Bars two, situated ventral to anchors, similar in shape, and with midportions bent toward, and articulated to, each other. Arms of anterior bar hollow and with well-defined articulating surfaces for attachment to anchors (Fig. B, 1). Posterior bar solid, articulations to anchors well defined (Fig. B, 5). A pair of knobs present on each bar at site of attachment with each other. Knobs of anterior bar apparently loosely attached to bar and each member of the pair with a strand of tissue reaching to the adjacent haptoral surface (Fig. B, 3). Proximal portion of each arm of posterior bar often with an accessory structure connecting it with the lateral portion of the haptor (Fig. B, 4). Greatest distance between arm tips of anterior bar 0.144 mm (0.100-0.216 mm); greatest distance between arm tips of posterior bar 0.137 mm (0.085-0.167 mm). Anchors thin, similar in shape, anterior pair larger; bases enormously developed, occupying the major portion of a frontal plane of the haptor, and limiting the greater portion of the haptoral outline (Fig. A, 8 and 11). Anchor shafts reduced in length and parts (former) of them apparently incorporated in the expanded anchor bases and each evident as a reinforced marginal portion of the base (Fig. D, 5). Separation of bases into roots barely indicated or absent in some specimens (Figs. A, B, D). Length of anterior anchors 0.179 mm (0.103-0.219 mm), greatest width 0.110 mm (0.063-0.144 mm); length of posterior anchors 0.160 mm (0.100-0.190 mm), greatest width 0.103 mm (0.076-0.140). Anchor wings well developed. Hooks similar in size and shape, fourteen in number, and situated around the margin of the haptor (Fig. A). Each hook consists of an oval base, a slender solid shaft, a sickle-shaped termination, and an opposable piece. Hook bases longitudinally ovate in outline and approximately the same length as the shaft. Hook lengths 0.017-0.021 mm.

Gonads ovoid in outline and located in rear half of body proper. Testis situated dorsoposterior to, and larger than, the ovary. Vagina on left body margin near the midportion. Copulatory complex consisting of a cirrus and accessory piece. Cirrus with a large base and a curved tapered terminal portion (Fig. C, 2). Accessory piece consisting of a thin expanded basal portion, which partially encloses the basal portion of the cirrus, and a solid recurved termination (Fig. C, 1). Cirrus length (distance between tip and most distal part of the base) 0.031 mm (0.024-0.039 mm); accessory piece approximately the same length as the cirrus. Vitellaria well developed and with arrangement as in the genus *Urocleidus* Mueller, 1934. The nature of the gut could not be determined from fixed material.

**Type hosts and localities:** Stump-knocker sunfish, *Eupomotis microlophus* (Günther, 1859) Englewood Ditch, Englewood, Fla.; Myakka River, State Park, East Sarasota, Fla.; Lake Okeechobee, Moore Haven, Fla.; and Everglades Canal, Naples, Fla. Bluegill sunfish, *Lepomis macrochirus* Rafinesque, 1819, Canal, North Everglades, Fla.

**Site of infestation:** Gills.

**Number of specimens used in this description:** Twelve.

**Type specimens:** Cotypes Nos. 36773-6 U. S. Nat. Mus., Washington, D. C.

In 1937 Summers described *Anchoradiscus triangularis* from the gills of *Lepomis symmetricus* Forbes, 1883, and assigned it to the genus *Actinocleidus* Mueller, 1937. Although specimens of this parasite were



not available for comparison, the writer secured several figures and notations from Dr. Summers and these together with the published description have shown this form to be a close relative of *Anchoradiscus anchoradiscus* and should be placed in the newly proposed genus.

The general morphology of species of *Anchoradiscus* is very similar to that of *Actinocleidus* forms. In addition, the haptor is discoidal and the bars are articulate in both genera. Differences between species of the genera are manifest in that those of *Anchoradiscus* possess (1) a thinner haptor, (2) anchors which lie parallel to the haptoral surfaces (dorsoventral) with their recurved points projecting laterally instead of ventrally as in *Actinocleidus* species, (3) bars which lie ventral to the anchors and attach to the lower lateral anchor surfaces instead of being located between the two pairs of anchors and attaching to them between the roots of the anchor bases as in *Actinocleidus* forms,<sup>2</sup> (4) hooks with large ovate bases instead of small spherical-to-ovate ones as in members of *Actinocleidus*, (5) anchor shafts which are much reduced and may be interpreted as wanting since the edge of the anchor base, which is in line with the vestigial shaft, is comparatively thick and probably includes a part or all of the anchor shaft, and (6) enormously developed anchor bases which when taken together (all four), occupy most of a frontal plane of the haptor. This last point presents a sharp contrast with reference to the relatively small anchor bases in species belonging to other genera of North American fresh-water TETRAONCHINAE.

It is very probable that the gut of *A. anchoradiscus* is bifurcate and posteriorly confluent as described for its closest relative (*A. triangularis*) by Summers (1937).

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<sup>2</sup> Attachment of the bars to the anchor bases has not been definitely established for all species of *Actinocleidus*. Attachment is thought to be made between the roots of the anchor bases or in this region in cases where the roots are absent.

## EXPLANATION OF PLATE

All drawings were made with the aid of a camera lucida at the designated magnifications.

## FIG. A (ventral view)

- |                       |                      |
|-----------------------|----------------------|
| 1. Head organs        | 7. Testis            |
| 2. Eye spots          | 8. Anterior anchor   |
| 3. Pharynx            | 9. Anterior bar      |
| 4. Copulatory complex | 10. Posterior bar    |
| 5. Vagina             | 11. Posterior anchor |
| 6. Ovary              | 12-15. Hooks         |

## FIG. B

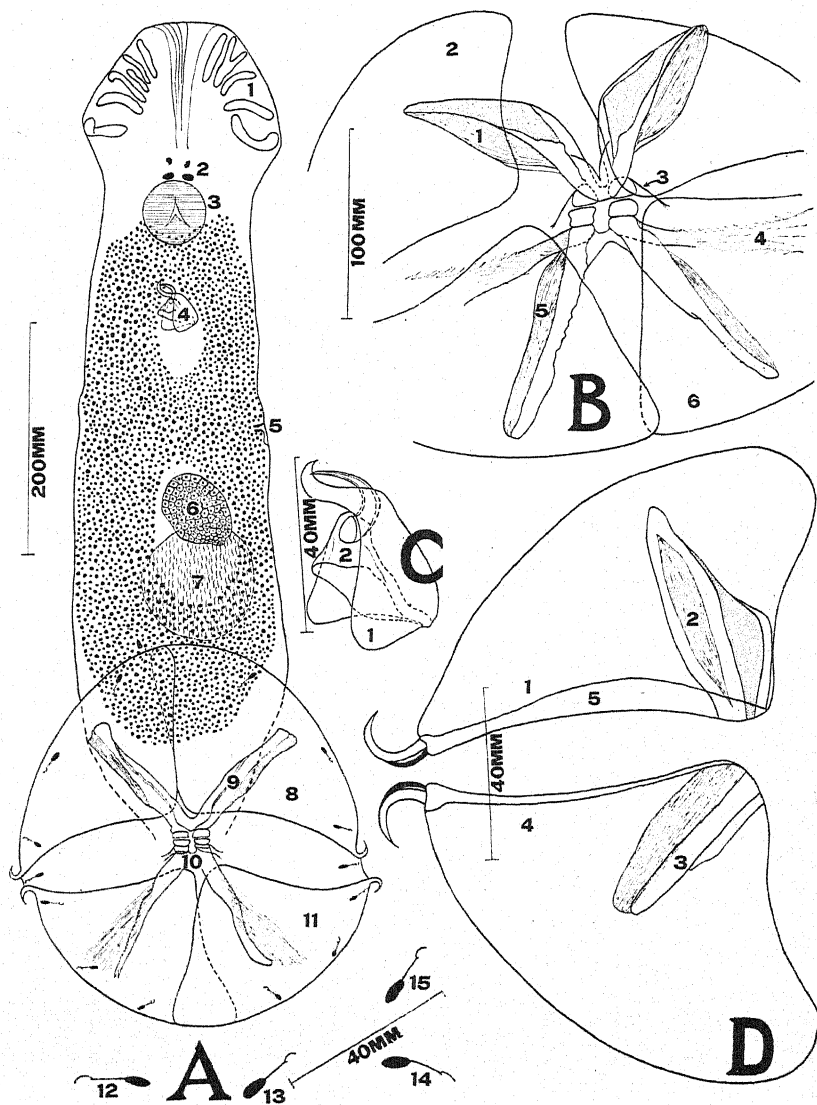
- |                               |                            |
|-------------------------------|----------------------------|
| 1. Anterior bar               | 4. Accessory bar-structure |
| 2. Anterior anchor            | 5. Posterior bar           |
| 3. Strand of tissue from knob | 6. Posterior anchor        |

## FIG. C

- |                    |           |
|--------------------|-----------|
| 1. Accessory piece | 2. Cirrus |
|--------------------|-----------|

## FIG. D

- |                                     |                          |
|-------------------------------------|--------------------------|
| 1. Anterior anchor                  | 3. Part of posterior bar |
| 2. Part of anterior bar             | 4. Posterior anchor      |
| 5. Thickened portion of anchor base |                          |





*DENDRITOBILHARZIA ANATINARUM* N. SP., A BLOOD  
FLUKE FROM THE MALLARD<sup>1</sup>

E. L. CHEATUM<sup>2</sup>

A new blood fluke of ducks belonging to the genus *Dendritobilharzia* Skrjabin, 1920, is here described, with a discussion on the occurrence of blood fluke eggs and their possible pathological significance to ducks.

Subfamily BILHARZIELLINAE Price, 1929

Price (1929) in his synopsis of the SCHISTOSOMATIDAE proposed the subfamily BILHARZIELLINAE to include the genera *Bilharziella* Looss, 1899, *Gigantobilharzia* Odhner, 1910, *Trichobilharzia* and *Dendritobilharzia* Skrjabin and Zakharow, 1920. Ejsmont (1929) added the genus *Pseudobilharziella*. The diagnosis given by Price for this subfamily includes a statement regarding character of the uterus, describing it as short and containing a single egg. This would exclude the species described here since it is long, coiled, and contains many eggs. All other structural characters agree with this subfamily diagnosis, and since the character of the uterus alone should not constitute a subfamily difference, the diagnosis is accordingly amended to read:

BILHARZIELLINAE Price, 1929, emend.

SCHISTOSOMATIDAE. Male and female similar in form, either flattened or thread-like. Suckers present or absent. Gynaecophoric canal absent or imperfectly formed. Paired intestinal ceca short, common cecum long, with or without lateral dendritic branches. Testes numerous, situated along course of common cecum. *Uterus may contain one or many eggs.*

Genus *Dendritobilharzia* Skrjabin, 1920

In examining the genera of BILHARZIELLINAE, the characters of *Dendritobilharzia* come most nearly corresponding to the form described here. The differences in character of the uterus, the number of eggs contained, and larger size of the female as compared to the male are not considered of sufficient significance to warrant establishing a new genus, therefore the generic diagnosis is amended to read:

*Dendritobilharzia* Skrjabin, 1920, emend.

BILHARZIELLINAE. Body of both sexes elongate and flattened. *Female larger or smaller than male.* Cuticle without spines or tubercules. Suckers absent. Digestive

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<sup>1</sup> Contribution from the Game Research Center, Bureau of Game, N. Y. S. Conservation Department.

<sup>2</sup> I wish to express my appreciation to Prof. George R. La Rue of the University of Michigan and to Dr. E. W. Price of the Bureau of Animal Industry for their kind advices, and to Dirck Benson and Douglas Beyea of the N. Y. S. Bureau of Game for identification of the ducks.



system similar to that in *Bilharziella*, common cecum long, zig-zag, provided with short, club-shaped or branched lateral ceca. Genital pore of male lateral, in anterior part of body. *Cirrus present*. Testes numerous, situated along common course of cecum, and extending from cecal union to end of body. Ovary spiral, between intestinal crura. *Uterus may contain one or many eggs*. Vitelline follicles numerous, along course of common cecum.

This genus contains two species, *D. pulverulenta*, first described by Braun (1901) as *Bilharziella pulverulenta* from *Anas platyrhynchos* collected from Africa, and *D. loossi* Skrjabin, 1924, from *Pelecanus onocrotalus* collected in the U.S.S.R. Skrjabin and Zakharow (1920) established the genus *Dendritobilharzia* for a new species of blood fluke from *Querquedula querquedula* to which they gave the name *Dendritobilharzia odhneri*. Later Skrjabin (1924) recognized *D. odhneri* as synonymous with Braun's *B. pulverulenta*. Price (1929) synonymized *D. odhneri* with *B. pulverulenta* and recognized *Dendritobilharzia* as the valid genus. Thus *Bilharziella pulverulenta* became *Dendritobilharzia pulverulenta* (Braun, 1901) Skrjabin, 1924. I have been unable to find reference to additional species of this genus. Brackett (1940) in listing the adult schistosomes from hosts which also occur in Wisconsin gives *Dendritobilharzia* sp. from *Melanitta deglandi*. This record was obtained by personal communication with L. R. Penner who apparently has not yet described this form.

The description of this genus has been based on a striking paucity of specimens. Braun described *D. pulverulenta* from a single male worm. In his description he did not mention the presence of a cirrus although his figure shows structure indicating its presence. Semenov (1927) claims to have found the female of Braun's *pulverulenta* and described it from a single specimen. Skrjabin described *D. loossi* from a single female worm, the male remaining unknown. Thus we have only one description of the male in this genus, that of *D. pulverulenta*. There is a possibility that Braun overlooked the presence of the cirrus in writing his description, and in amending the generic diagnosis I have included the presence of a cirrus to conform with the species described here and Braun's figure of *D. pulverulenta*.

*Dendritobilharzia anatinarum* n. sp.

(Figs. 1, 2)

Two female and five male worms have been removed from the dorsal aortae of two mallard ducks, *Anas platyrhynchos platyrhynchos* Linnaeus. These birds were hatched and reared at the Game Research Center of the New York State Bureau of Game, located near Delmar, N. Y.

*Male*. Body similar in shape to female but smaller, 6.2 mm long by 0.60–0.75 mm wide; esophagus 0.427 mm long, intestinal crura united posteriorly 1.08 mm from esophageal bifurcation; testes from 120–130 in number, extending from union of intestinal crura to posterior end of body; vesicula seminalis elongate, arranged roughly in a V-shape, about 0.26 mm long, median between intestinal crura; genital pore dextral, 1.20 mm from anterior end of body; cirrus well developed, distal end bulbous.

*Female.* Body flattened, elongate, 8.0 mm long by 0.89–1.01 mm wide; suckers absent; oral opening sub-terminal, esophagus 0.621 mm long, intestinal crura united posteriorly 1.55 mm from esophageal bifurcation, common cecum with lateral dendritic branches, proceeding in zig-zag course to posterior end of body; ovary long, tubular, arranged in close compact loops, between intestinal crura, length and width of mass about equal, 0.525 mm; receptaculum seminis immediately posterior to ovary and median, 0.078 mm by 0.272 mm; uterus in close compact loops filling anterior half of space between intestinal crura, then proceeding anteriorly in irregular course, right of esophagus to median genital pore, 0.350 mm from anterior end of body; unpaired vitelline duct proceeding anteriorly left of ovary to oötype at level of anterior end of ovary, vitellaria filling body along course of common cecum; eggs numerous, spherical, 0.060–0.065 mm diameter, with thin, elastic, transparent shells without spines, and undeveloped when deposited (Fig. 3).

*Host:* *Anas platyrhynchos*.

*Location:* Dorsal aorta.

*Locality:* Delmar, New York.

*Type:* U. S. N. M. Helm. Coll. No. 39895.

The species *D. anatinarum* differs from the 2 other described species in the following main characters: 1. Uterus long, contains many eggs. The other species have short uteri with single eggs. 2. The female is larger than the male, whereas in the other species where both sexes are known the male is the larger.

#### DISTRIBUTION AND PATHOLOGY

During the past winter (1939–40) numerous ducks have been received at the Laboratory of Wildlife Pathology for autopsy. Many of these were found sick or dead on the State Game Farms or in the wild. Malnutrition as a result of hard winter conditions was at first suspected. Empty stomachs, emaciation, atrophic livers, blood-stained serous exudate in the body cavity, and enteritis were the usual findings. These were not with the exception of enteritis, incompatible with the general picture of malnutrition. Scrapings from intestinal linings revealed the presence of numerous spindle-shaped eggs (Fig. 4) in the mucosa and sub-mucosa. Pieces of liver and kidney pressed between two slides revealed more of the same type of eggs. Later blood flukes were found in the dorsal aorta of a mallard dying from a severe enteritis involving the lower intestine. Scrapings from the inflamed mucosa showed numerous spindle-eggs and great numbers of spherical eggs as were found being deposited by the worm described here.

Following the association between duck mortalities and the presence of blood fluke eggs, all ducks coming to the laboratory were checked for the presence of blood flukes. Microscopic examination of scrapings from the rectal and cecal mucosae for the presence of eggs was relied upon for the incidence of flukes. Table 1 shows this incidence.

It will be noted that the incidence of blood flukes is much higher in the black ducks reared on Game Farms than in those collected from the wild. This is to be expected since the constant use of game farm ponds

over a period of years and continuous habitation throughout the seasons would tend to concentrate the incidence of infection in the intermediate snail hosts. I haven't sufficient mallards from the wild to make similar comparison but the high incidence of blood flukes in the game farm birds is comparable to that of the black ducks. Incidence in the seven other species comes from birds found dead or so weak they were unable to escape capture. This selective collecting may preclude fair judgment on the actual incidence in the wild, therefore the high incidence among the canvas-back ducks may be misleading.

TABLE 1\*

	Species	No. exam.	Eggs pres.
Game Farm .....	<i>Anas p. platyrhynchos</i>	23	18 (2)
Wild .....	"	1	1
Game Farm .....	<i>Anas rubripes</i>	9	7 (1)
Wild .....	"	25	4
" .....	<i>Spatula clypeata</i>	2	1
" .....	<i>Dasula acuta</i>	1	1
" .....	<i>Nettion carolinense</i>	1	1
" .....	<i>Nyroca collaris</i>	1	1
" .....	" <i>calisineria</i>	6	5 (1)
" .....	" <i>marila</i>	1	1
" .....	<i>Glaucionetta clangula</i>	2	1
	Totals	72	41 (4)

\* Data taken from specimens from Jan. 18 to June 10, 1940.

All of the forty-one (41) positive cases showed spindle eggs, and in four (4) instances, indicated in parentheses, the spherical type was also present.

The life histories of these blood flukes are being investigated at the Game Research Center preliminary to the problem of determining their pathological significance to ducks. There is good evidence at present that the eggs of *D. anatinarum* when working through the intestinal mucosa in large numbers cause hyperemia, petechial hemorrhages and may result in necrotic degeneration of the epithelium, sub-mucosa and circular muscle layer. This necrosis may result from bacterial invasion of the lesions caused by the fluke eggs. Experiments are planned to determine more accurately the role of blood flukes in producing this enteritis.

#### SUMMARY

A blood fluke, *Dendritobilharzia anatinarum* n. sp. is described from the common mallard, *Anas platyrhynchos* Linnaeus, with a modification of the subfamily diagnosis of BILHARZIELLINAE and a modification of the generic diagnosis for *Dendritobilharzia*. A table on the incidence of blood fluke eggs in nine species of ducks, including both the diving and surface-feeding groups, is given along with remarks on the lesions associated with their occurrence.

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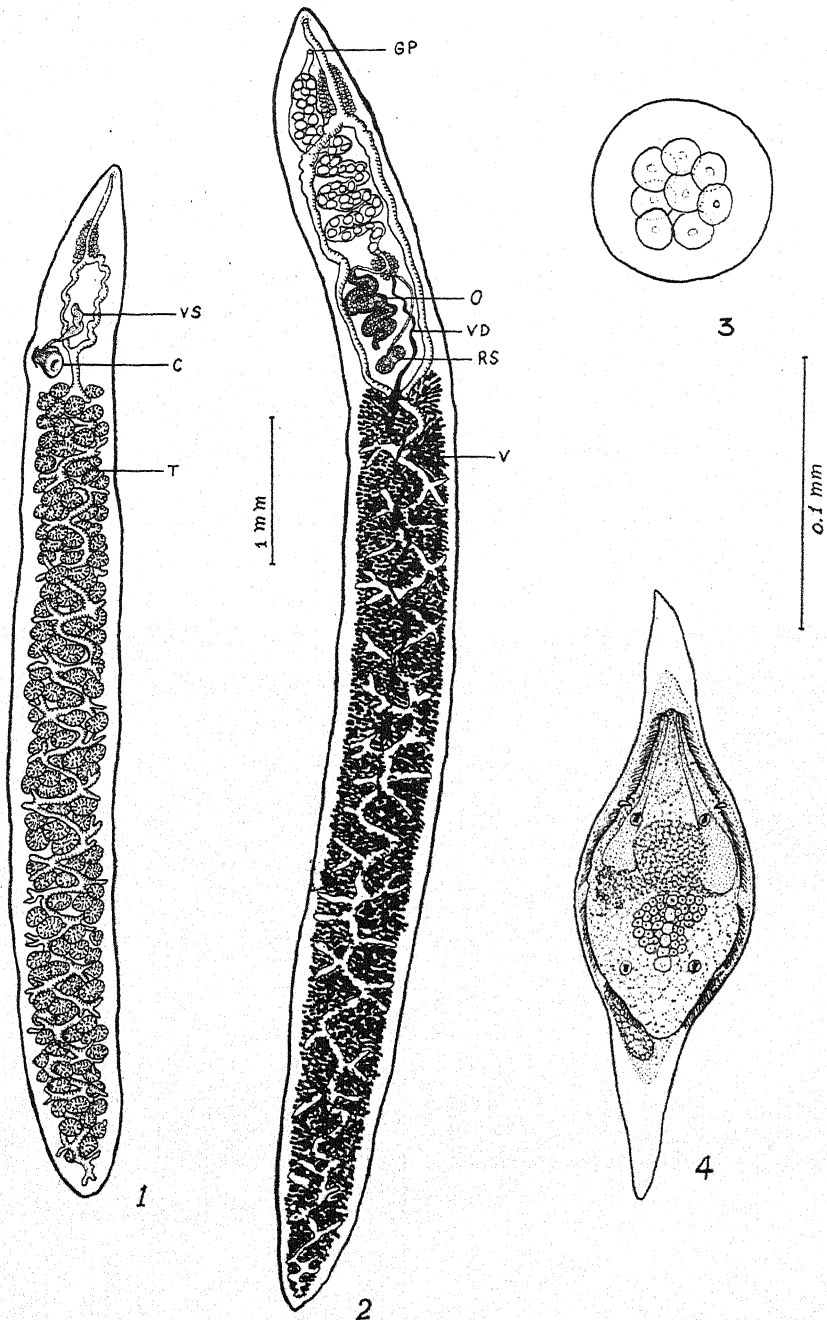
## EXPLANATION OF PLATE, p. 170

FIG. 1. *Dendritobilharzia anatinarum*, adult male, ventral aspect: vs, vesicula seminalis; c, cirrus; t, testes.

FIG. 2. Adult female, ventral aspect: gp, genital pore; o, ovary; vd, vitelline duct; rs, receptaculum seminis; v, vitellaria.

FIG. 3. Egg when deposited. Spherical type.

FIG. 4. Unidentified egg of spindle type with well developed miracidium.



2  
*Dendritobilharzia anatinarum*



GRYPORHYNCHUS TETRORCHIS, A NEW DILEPIDID CESTODE FROM THE GREAT BLUE HERON\*

WILLIAM C. HILL

A great blue heron, *Ardea herodias herodias* Linnaeus, collected near Cromwell, Oklahoma, during the summer of 1938 was found to harbor several specimens of an apparently new tapeworm described herein as *Gryporhynchus tetrorchis*.

The worms were fixed in hot Bouin's fluid and stained with borax carmine. All studies and measurements were made on mounted specimens.

*Gryporhynchus tetrorchis* n. sp.

(Figs. 1-4)

*Description:* Largest strobila having a scolex, 15.8 mm long, with 240 proglottids—none of them gravid. Largest headless fragment, 22 mm long, with 195 segments—some of them partially gravid. Strobila slender, subcylindric, with moderately serrate margins. Proglottids all wider than long; the mature ones trapezoidal, about 130  $\mu$  long, 220  $\mu$  wide anteriorly and 273  $\mu$  posteriorly; gravid segments similar in shape to, and scarcely larger than, mature ones. Genital primordia first discernible at about 200  $\mu$ , in segment No. 38, and mature at 10.6 mm, in No. 218, from front end.

\* Scolex 200-253  $\mu$  long by 213-286  $\mu$  wide. Rostellum comprises a distinctly outlined somewhat hyaline, medulla (83-93  $\times$  71-78  $\mu$ ) and a less well defined, apparently muscular, cortex (104-150  $\times$  75-98  $\mu$ ); armed with 20 hooks in two rows. Front hooks 60-72  $\mu$  long, 30-36  $\mu$  from tip of handle to tip of guard, 36-41  $\mu$  from tip of guard to tip of blade; corresponding measurements of back hooks—35-42, 18-21, 21-23,  $\mu$ . Neck very short.

Dorsal and ventral excretory canals approximately equal in size. Relationship of genital ducts to excretory vessels not observed. Genital pores unilateral, sinistral, pre-equatorial, and small as compared with the atria. Genital atrium in a mature segment, 50  $\mu$  deep and 38  $\mu$  in longitudinal diameter (exclusive of special pouches mentioned below); provided with 4 very prominent claw-like genital spines about 21  $\mu$  long, located 2 in each of 2 special pouches situated one in front of, and one behind, the vaginal pore.

Testes, 4 in number (no variation in number observed), spheroidal, 45-68  $\mu$  in diameter when mature, grouped in central portion of segment posterodorsal to female organs; still recognizable in gravid proglottids, arranged in a transverse row along posterior side. Cirrus pouch, 75  $\times$  45  $\mu$ , ellipsoidal, extends mediosubanteriad to near middle of segment, contains cirrus in distal, and a loop of vas deferens in proximal, portions. Cirrus narrowly ellipsoidal, 41  $\times$  18  $\mu$ , armed with numerous fine, porally directed, hair-like spines. Vas deferens loosely coiled anteroventrally in median portion of segment. Vasa efferentia not observed.

Mature ovary 112  $\mu$  in maximum diameter, transversely elongate, bilobate—the right and left lobes being connected by a slender elongate isthmus; dextral lobe usually further subdivided into anterior and posterior lobules. Vitellarium broadly ellipsoidal, transversely elongate, behind ovary near center of proglottid, about 37  $\mu$  in transverse diameter. Mehlis' gland situated dorsally between vitellarium and left lobe of ovary. Uterus at first a small indistinct sac; later it becomes roughly U-

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\* Contribution, No. 80, from the Zoological Laboratory, Oklahoma Agricultural and Mechanical College, prepared under the direction of R. Chester Hughes.

shaped, the limbs being directed backward and variously marked by constrictions. Proximal portion of vagina thick walled and dilated near the atrium, located immediately dorsal to cirrus pouch; distal end of vagina expanded as a thin-walled spheroidal seminal receptacle about  $23\ \mu$  in diameter.

*Host*: *Ardea herodias herodias* Linnaeus, 1758.

*Habitat*: Small intestine.

*Locality*: Cromwell, Oklahoma.

*Cotype specimens*: In toto mounts of two entire strobilae and numerous fragments in U. S. Nat. Mus. Helm. Coll.

The genus *Gryporhynchus* von Nordmann, 1832, now contains 4 known species—(1) *G. pusillus* von Nordmann, 1832 (synonym, *Taenia macropeos* Wedl, 1855); (2) *G. cheilancristrotus* (Wedl, 1855) Ransom, 1909; (3) *G. macrorostratus* (Fuhrmann, 1907) Ransom, 1909; and (4) *G. tetrorchis* n. sp. *G. macrorostratus* was taken from a passeriform host, the others from CICONIIFORMES. The new species is apparently the first to be reported from the western hemisphere. Used in this study were the compilatory descriptions of Joyeux and Baer (1936) of all 3 of the old species, the original account of Fuhrmann's species, and a description by Meggitt (1931) of worms identified reservedly as "*G. macropeos* (Wedl, 1856)."

The new species differs from all the others in having fewer testes, a relatively shorter cirrus pouch, a new world distribution, and, apparently, in the U-shape of the gravid uterus. Further, *G. tetrorchis* is considerably larger than *G. pusillus*, considerably smaller than *G. cheilancristrotus*, and unlike *G. macrorostratus* in having 4 instead of 2 genital spines in the atrium. According to diagnoses of *Gryporhynchus* by Ransom (1909) and Meggitt (1924) the testes are 6-8 in number; Meggitt (1931), however, found 5 in his above-mentioned specimens of "*G. macropeos*."

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## EXPLANATION OF PLATE, p. 174

All figures concern *Gryporhynchus tetrorchis* n. sp. and all were drawn with the aid of a camera lucida, minor details added free-handedly.

## ABBREVIATIONS

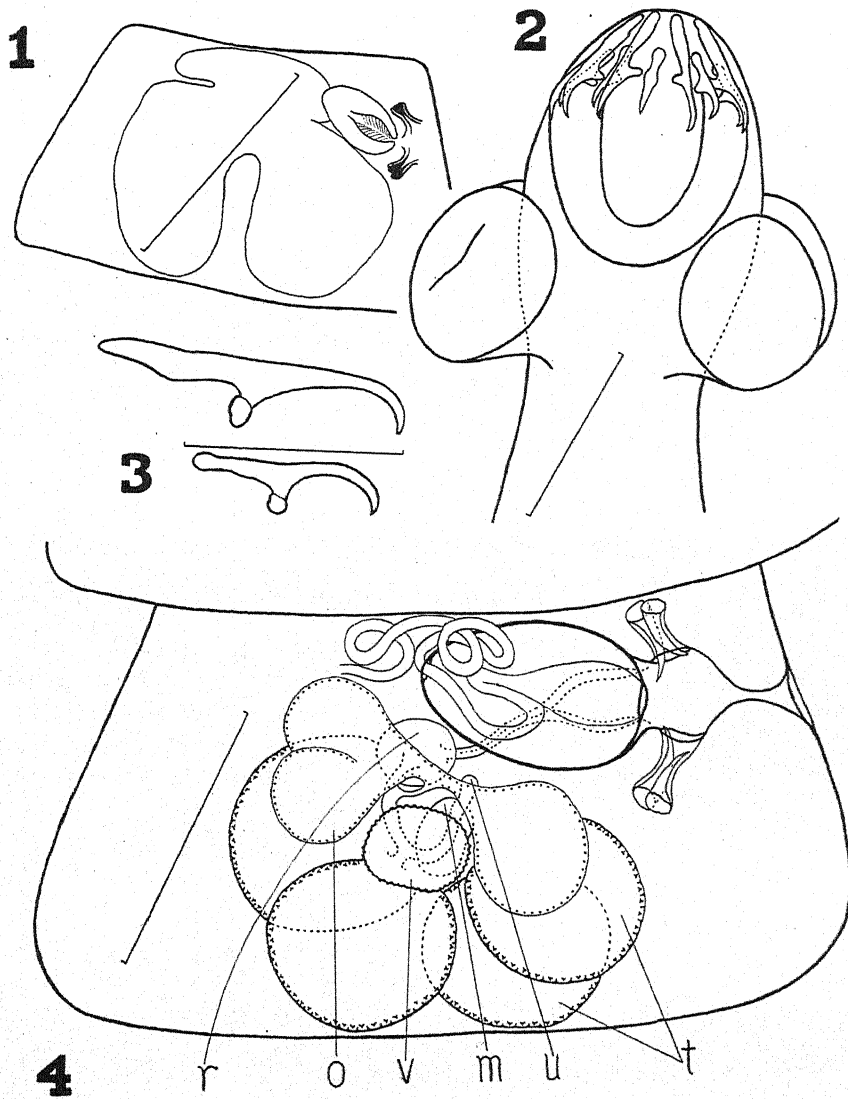
in—Mehlis' gland  
o—ovary  
r—seminal receptacle  
t—testes  
u—uterus  
v—vitellarium

FIG. 1. Partially gravid proglottid. Note hair-like spines in cirrus and U-shape of uterus. Scale = 200  $\mu$ .

FIG. 2. Scolex with rostellum everted. Scale = 100  $\mu$ .

FIG. 3. Rostellar hooks, front and rear. Scale = 50  $\mu$ .

FIG. 4. Mature proglottid, ventral view, somewhat diagrammatic. Scale = 100  $\mu$ .



*Gryporhynchus tetrorchis*

## HELMINTHS OF MUSKRATS IN SOUTHEAST TEXAS

ASA C. CHANDLER

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In connection with an ecological study of native fur animals in Southeast Texas conducted by Mr. Dan W. Lay, Regional Game Manager, Texas State Game, Fish and Oyster Commission, 36 muskrat carcasses were made available for parasitological examination. Sixteen of the animals were collected from Big Hill Bayou, near Port Arthur in Jefferson Co., and 20 were collected a few miles northeast of High Island in Chambers Co.

As will be seen from the accompanying table there were some striking differences in the parasite fauna of the rats from these two localities. Mr. Lay states that both localities are meadow-like marshes similar in general appearance, and have about equal muskrat populations. In the Jefferson Co. locality, however, the water is only slightly brackish, fresh enough for rice cultivation and to support a good growth of *Cabomba caroliniana*, whereas in the Chambers Co. locality the water is tidal and brackish as indicated by the presence of *Ruppia maritima*. The absence of the filarial worm, *Litomosoides carinii*, from the Chambers Co. muskrats may possibly be due to failure of the unknown intermediate host to breed in brackish water. The presence of *Nudacotyle novicia* in the brackish water area and not in the area with fresher water, however, probably cannot be accounted for in a similar manner, since this fluke has hitherto been reported from an inland locality.

The helminths of muskrats were first extensively studied by Barker (1915) in Nebraska. Since then one or a few specimens of this host have been examined in various parts of this country and in Europe, and a considerable number of new species of helminths added. The localities represented include the Great Lakes area (Barker, 1916; Price, 1931; Ameel, 1932; Olivier, 1938; Dikmans, 1935; Olsen, 1939), Maryland (Price, 1931; Smith, 1938), Great Britain (Warwick, 1936), and Russia (Schulz et al, 1933).

The parasitic fauna of the Texas muskrats is notably different from that recorded in any of these localities. Only two of the 22 species of flukes hitherto recorded from muskrats, and one new species, were found, namely: *Echinochasmus schwartzi*, hitherto recorded once, from Maryland, by Price, 1931; *Nudacotyle novicia*, hitherto recorded once, from Minnesota, by Barker, 1916; and *Phagicola lageniformis* n. sp. Of these *Echinochasmus schwartzi* was the commonest helminth, both from the standpoint of incidence and of numbers, in hosts from both localities.

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TABLE 1.—*Helminth parasites of muskrats in southeast Texas*

Parasites	Jefferson Co. 16 rats examined		Chambers Co. 20 rats examined	
	No.	%	No.	%
Trematodes				
<i>Echinochasmus schwartzi</i> .....	11	69	10	50
<i>Phagicola lageniformis</i> .....	0	0	1	5
<i>Nudacotyle novicia</i> .....	0	0	6	33
Nematodes				
<i>Strongyloides ratti</i> var. <i>ondatrae</i>	0	0	1	5
<i>Longistriata dalrymplei</i> ? .....	0	0	1	5
<i>Rictularia ondatrae</i> .....	1	6	2	10
<i>Litomosoides carinii</i> .....	4	25	0	0
Cestodes .....	0	0	0	0

*Nudacotyle novicia* occurred in 6 of 20 rats collected in Chambers Co., and in considerable numbers, but was entirely absent from the 16 rats collected in Jefferson Co. *Phagicola lageniformis* n. sp. was found in moderate numbers only in one rat, from Chambers Co.

Four species of nematodes were collected, of which three are definitely new from this host. One, *Rictularia ondatrae* n. sp., was found in three of the 36 rats examined, and was represented in both localities. *Strongyloides ratti* var. *ondatrae* n. var. was found in a single rat from Chambers Co., in small numbers. In the same rat were found two female specimens of a heligmosome which could not be positively identified in the absence of males, but which is probably *Longistriata dalrymplei* Dikmans, 1935. In 4 of the 16 rats from Jefferson Co., but in none from Chambers Co., were found adults and microfilariae of *Litomosoides carinii* (Travassos, 1919), a species common in cotton rats, *Sigmodon hispidus*, in Texas and Mexico. No tapeworms, either larvae or adults, were encountered.

Descriptions of the new species and variety follow.

*Phagicola lageniformis* n. sp.

(Figs. 1-2)

Very small flask-shaped flukes; posterior portion almost spherical, preacetabular portion drawn out into a slender neck about as long, or longer, than the posterior portion. Length 0.45 to 0.63 mm; maximum diameter of posterior portion 0.23 to 0.26 mm; diameter of neck at narrowest point only 45 to 65  $\mu$ . Dorsal lip well developed, rounded. Oral sucker 45 to 55  $\mu$  in diameter, surrounded by a crown of 18 spines, 16 of them in a single circle, two situated more posteriorly on dorsal side; spines 18 to 20  $\mu$  long, all approximately equal in size. Oral diverticulum broad at base, tapering to a narrow and usually twisted end; length 90 to 140  $\mu$ , in most specimens reaching only from half to two-thirds distance from oral sucker to pharynx. Pharynx 30 to 33  $\mu$  wide and 33 to 38  $\mu$  long, its anterior rim situated 120 to 220  $\mu$  from anterior end, depending upon degree of contraction; esophagus very short; ceca wide, not traceable behind level of acetabulum, where they probably end. Acetabulum 40 to 50  $\mu$  in diameter, not obviously sunk in genital sinus, situated at or slightly behind middle of body length.

Testes situated at or near posterior end of body, usually transversely elongated, varying from 60 by 70  $\mu$  to 35 by 90  $\mu$ . Ovary slightly smaller than testes, situated in front of right testis. Seminal receptacle very large, median, in front of testes and at left of ovary. Vitellaria consist of two groups of usually 7 follicles each, situated at

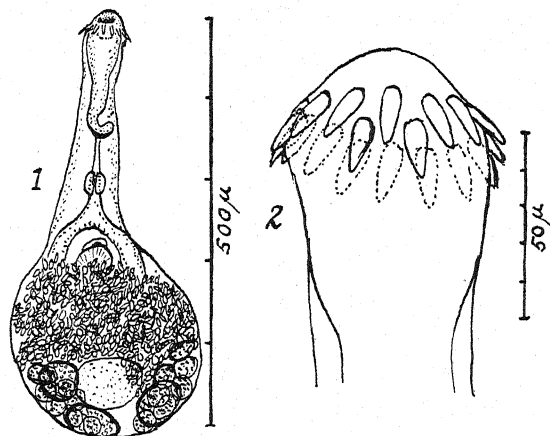


Fig. 1. *Phagicola lageniformis* n. sp. Ventral view.

Fig. 2. *Phagicola lageniformis* n. sp. Cephalic crown of spines.

sides of posterior part of body, partly overlying the testes and extending forward to the level of the ovary. All available space between posteriorly situated reproductive organs and acetabulum occupied by irregular coils of the uterus filled with eggs. Eggs 20 to 21 by 10 to 12  $\mu$ .

*Host*: Muskrat.

*Habitat*: Intestine.

*Locality*: Southeast Texas.

*Type specimen*: Deposited U. S. N. M., Helm. Coll.

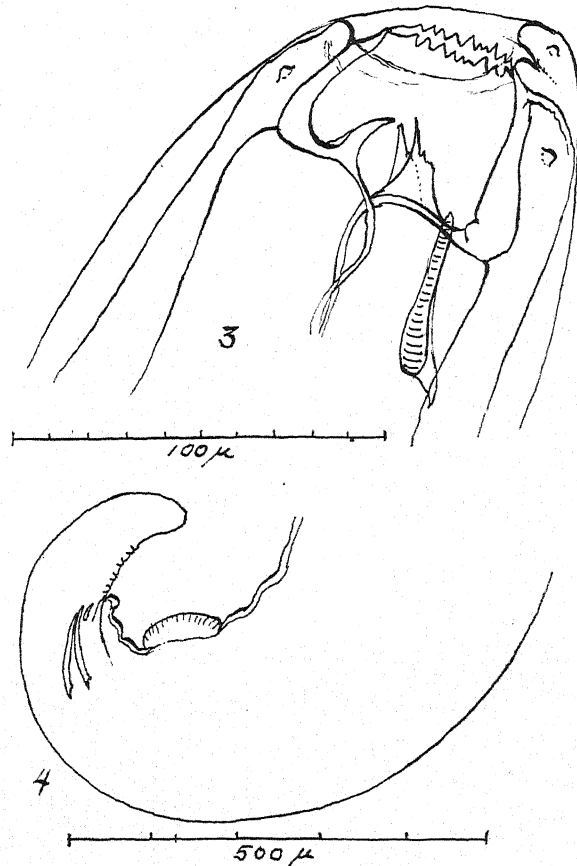
This species resembles *P. nana* (Ransom, 1920) and *P. angrensis* (Travassos, 1916) in the number and arrangement of the oral spines, but differs from *nana* in shape of body, size of oral diverticulum, and size of spines, and from *angrensis* in shape of body and in length of the prepharyngeal region, which in *angrensis* is very short, resulting in the oral diverticulum reaching beyond the pharynx in that species.

*Rictularia ondatrae* n. sp.

(Figs. 3-4)

Females large coarse worms, males relatively very small. Mouth terminal, but with a liplike projection of the ventral wall; buccal capsule heavily chitinized with three sharp teeth, jagged in some specimens, at the bottom, and a ring of about 18 denticles on its rim. Two pairs of papillae on the ventral side, one pair on dorsal side. Anterior end of first comb at level of bottom of capsule.

Females up to 28 or 30 mm long, with a maximum diameter, behind middle of body, of 850 to 900  $\mu$ . Mouth cavity about 60  $\mu$  across at anterior end, widening out to about 90  $\mu$  at bottom; about 85  $\mu$  deep. Diameter of head where cuticle bulges behind mouth capsule about 240  $\mu$ . Combs 24 or 25 to end of esophagus, 32 to vulva; total number of combs and spines 73 to 75. Combs in esophageal region contiguous, the backward-projecting spines very small, the pointed anterior ends of the bases much more conspicuous; cuticular processes in vulvar region transitional between combs and spines; projecting part of largest spines, about 1 mm behind vulva, 50  $\mu$  long, from here gradually decreasing in size and becoming spaced farther and farther apart, the last spines extremely small, over 1 mm apart, and ending about 2.5 to 3 mm from end of tail. Esophagus 3.2 to 3.5 mm long. Vulva 0.5 to 1 mm behind end of

FIG. 3. *Rictularia ondatrae* n. sp. Head of male.FIG. 4. *Rictularia ondatrae* n. sp. Caudal end of male.

esophagus. Vagina turns posteriad, and bifurcates about 300 to 400 μ behind vulva. Tail conical, rounded at tip, 500 to 630 μ long. Eggs 50 to 52 by 37 to 38 μ.

Males 6 to 7 mm long, with maximum diameter of about 420 μ. Esophagus 1.5 mm long. Mouth cavity 32 μ across at opening, 50 μ across at bottom, 40 μ deep. Combs contiguous for about one-third of body length, then becoming more and more widely spaced, but only about 200 μ apart, point to point, even at posterior end of body, and not decreasing in size very much. Last spines about 350 μ anterior to anus. Total number of combs and spines 52. Anus about 150 μ from tip of tail. Spicules nearly equal, one 110 μ long, the other about 98 μ; gubernaculum 20 μ long. A large median papilla just anterior to cloaca; no preanal papillae seen; four pairs of postanal papillae about equally spaced, last one about midway between cloaca and tip of tail. One fanlike expansion of the cuticle on midventral line, beginning 90 to 100 μ anterior to cloaca, 90 μ long and 30 μ broad.

*Host:* Muskrat.

*Habitat:* Intestine.

*Locality:* Southeast Texas.

*Type specimen:* Deposited U. S. N. M., Helm. Coll.

As noted by Hall (1913) the species of *Rictularia* fall into two well-

TABLE 2.—The following table shows the distinctions between species of *Rictularia* so far described from North America

	<i>splendida</i> Hall, 1913	<i>coloradensis</i> Hall, 1916	<i>scalopsis</i> Goodrich, 1932*	<i>halli</i> Sandground, 1935	<i>onychomis</i> Cuckler, 1939	<i>ondatrae</i> Chandler, 1940
Length .....	♀ 8-10.5 mm ♂ 4.8 mm	♀ 9-10 mm ♂ 3 mm	♀ 5.4 mm ♂ unknown	♀ 47-70 mm ♂ 3.7-7.8 mm	♀ 24-42 mm ♂ Unknown	♀ 25-30 mm ♂ 7 mm
Total, combs and spines, ♂ .....	108-109	42	Unknown	37-38	Unknown	52
Same, ♀, prevulvar and postvulvar	55 + 83	31 + 33 +	28 + ?	25-28 + 5-10	33 + 23-27	32 + 41-43
Position of vulva relative to end of esophagus .....	Just behind	Opposite	2 mm behind	Just behind	Anterior	0.5 to 1 mm behind
Position of first comb relative to buccal capsule .....	Own length behind	16 $\mu$ behind	93 $\mu$ behind	Just behind	100 $\mu$ behind	Reach to it
Length of spicules .....	207 $\mu$ , 207 $\mu$	145 $\mu$ , 180 $\mu$	Unknown	40-50 $\mu$ , 95-100 $\mu$	Unknown	98 $\mu$ , 110 $\mu$
Ventral fans (♂) .....	8	None	Unknown	4 or 5	Unknown	One
Host .....	Coyote	Chipmunk	Mole	Chipmunk	Grasshopper mouse	Muskrat

\* *R. citelli* McLeod, 1933, from two species of *Citellus* in Manitoba is not sufficiently well described to distinguish it from *R. scalopsis* Goodrich, 1932. The females of *R. citelli* are 30-60 mm long, with 23 prevulvar spines; a single male was 14 mm long, had equal spicules 117  $\mu$  long, and no ventral fans.

defined groups: (1) those from carnivores, the females of which have over 100 pairs of combs and spines, of which the majority are combs, and in which there is no well-defined transition from combs to spines in the region of the vulva, and (2) those from rodents, insectivores and bats, in the females of which there are less than 100 combs and spines, and a fairly sharp transition from one to the other in the region of the vulva. In many species the male is unknown, and this is true of two of the five previously known forms from North America (*R. scalopsis* and *R. onychomis*). Fortunately there are good characters for separating the females, and Cuckler (1939) devised a key based on this sex alone, though he omitted *R. halli* Sandground, 1935. *R. ondatrae* differs from all the other North American forms of the rodent-insectivore-bat group in having a total of over 70 combs and spines, and 32 prevulvar combs. It differs widely from the forms in which males are known in the size of the spicules, and in the number of ventral fans anterior to the cloaca. Other differential characters are shown in the accompanying table. The present species comes nearest to *R. scalopsis* Goodrich, 1932, but is smaller, has a much less prominent ventral lip in front of the oral capsule, has the first comb more anterior in position, has the vulva nearer to the end of the esophagus, and has a larger number of prevulvar combs. The total number of combs and spines in *R. scalopsis* is not given, and the male is unknown.

*Strongyloides ratti* var. *ondatrae* n. var.

Only a few specimens of this parasite were found. It resembles *S. ratti* in most respects but is longer and much more slender than the typical form as described by Sandground (1925). The variety from the muskrat is 4 mm long with a diameter of only 33  $\mu$ , 1/120 of the length, whereas the size of typical *S. ratti* as given by Sandground is 1.85 to 3.03 mm with a diameter of 40  $\mu$ , 1/60 of the length. In *ondatrae* the anus is 55 to 60  $\mu$  from the posterior end, and the vulva divides the body 2.5:1.5. The esophagus is 1 mm long, and the eggs measure 45 by 25  $\mu$ .

*Host*: Muskrat.

*Habitat*: Intestine.

*Locality*: Southeast Texas.

SUMMARY

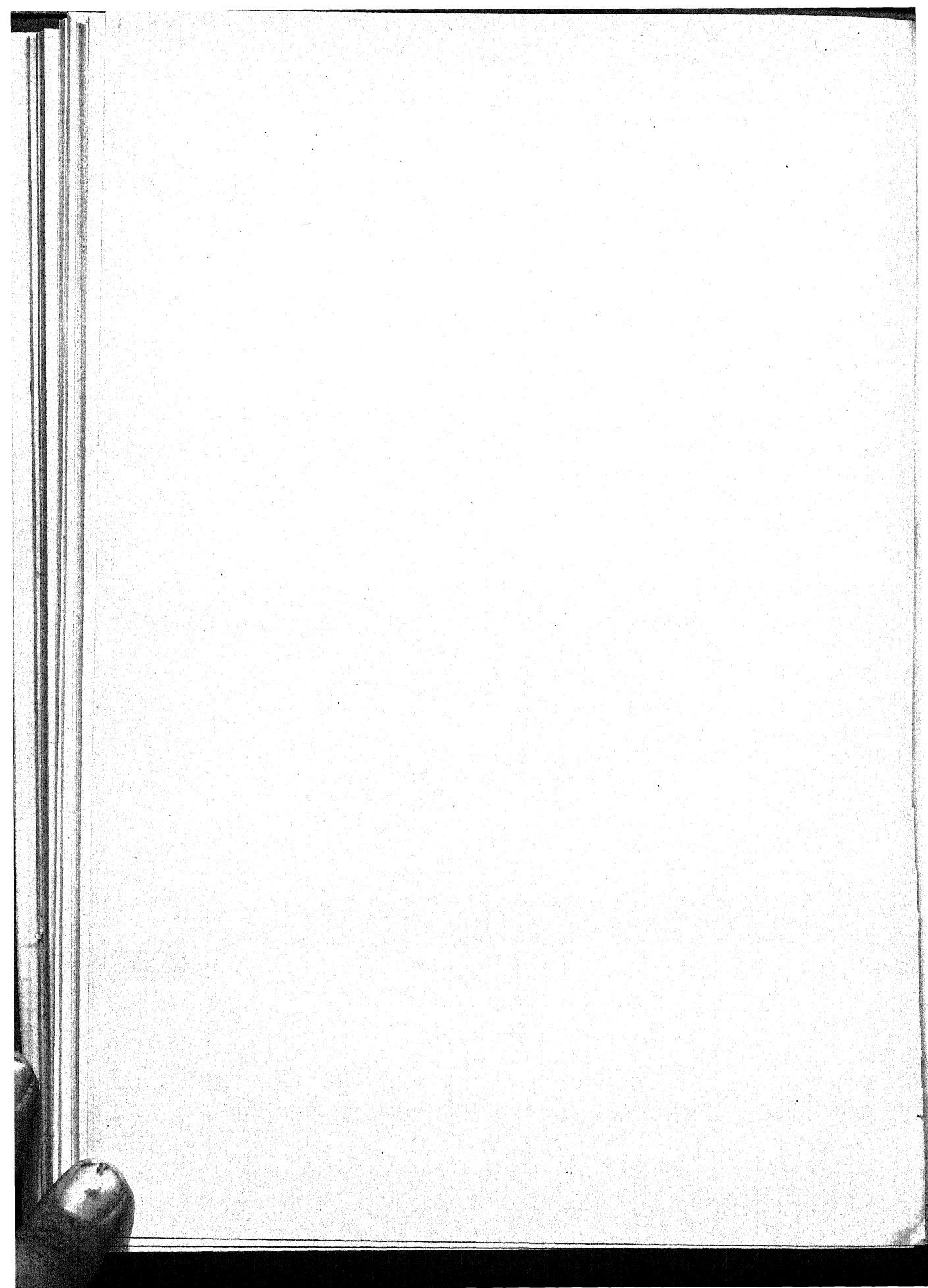
Thirty-six muskrats collected in southeast Texas yielded 3 species of trematodes, four of nematodes, and no cestodes. One trematode, *Phagicola lageniformis*, is new; of the nematodes, *Rictularia ondatrae* is new; *Strongyloides ratti* var. *ondatrae* is a new variety; and *Litomosoides carinii* is a new host record.

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## RESEARCH NOTES

### TWO NEW TREMATODES FROM THE BONITO, *SARDA SARDA*, IN THE GULF OF MEXICO

A specimen of bonito, *Sarda sarda*, taken off the Texas coast near Freeport, yielded two new species of trematodes, one a gasterostome of the genus *Rhipidocotyle*, the other a hemiurid of the genus *Sterrhurus*. Descriptions of them follow:

#### *Rhipidocotyle angusticollis* n. sp.

(Figs. 1-3)

Body elongate, broadest at about level of ovary; anterior portion constricted to a narrow neck just behind sucker. Anterior end of very peculiar shape with two hornlike projections on each side dorsally, a pair of lobes overhanging the sucker ventrally, and the sucker itself projecting ventrally and posteriorly like a chin. Length 1.2 to 1.4 mm; maximum diameter 300 to 420  $\mu$ ; diameter of neck about 65  $\mu$ , its depth only about 30  $\mu$ ; diameter across dorsal horns about 170  $\mu$ ; length of sucker about 110  $\mu$ . Pharynx about 60  $\mu$  in diameter; intestinal sac about 190  $\mu$  long and 135  $\mu$  wide, situated behind middle of body. Ovary at level of posterior portion of intestinal sac, at right, about 65 to 70  $\mu$  in diameter; testes one behind the other, posterior to the ovary, about 120 to 130  $\mu$  in diameter. Cirrus sac about 400  $\mu$  long; copulatory bursa about 125  $\mu$  in diameter. Vitellaria in two clusters of about 15 follicles each, the follicles about 40  $\mu$  in diameter, situated at level of anterior part of intestinal sac and anterior to it; vitelline ducts unite at level of junction of two testes. Uterus occupies available space behind intestinal sac and to left of testes; eggs about 21 to 22  $\mu$  long and 14 to 16  $\mu$  broad.

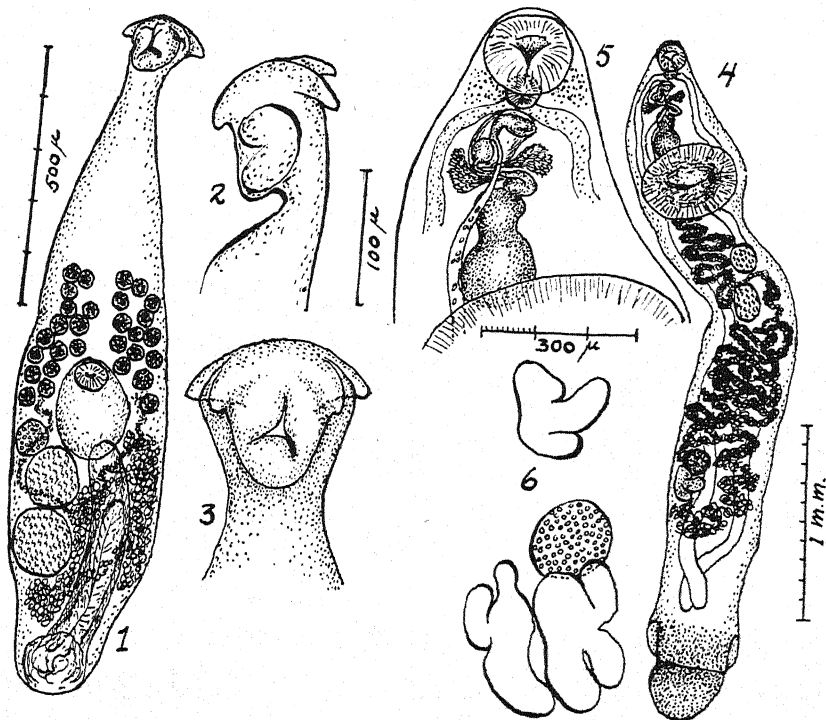
This species differs from all of the nine members of the genus hitherto described from marine fishes (see Eckmann, 1932; Chandler, 1935; Manter, 1940) by the very slender neck, deeply indented ventrally behind the anterior sucker, the posterior edge of which projects chinlike, and in the peculiar shape of the cephalic hood, with its prominent horns projecting dorso-laterally.

#### *Sterrhurus texanus* n. sp.

(Figs. 4-6)

Body elongate, cylindrical for most of its length, but bulging markedly at level of ventral sucker, and tapering anterior to it; retractile appendage only about 1/7 of body length. Length 3.25 to 3.6 mm; breadth behind ventral sucker about 0.5 mm, depth about 0.4 mm. Retractable appendage rounded, about 0.5 mm long. Oral sucker 150 to 180  $\mu$  in diameter; ventral sucker very large, over three times as broad as oral sucker measuring 550  $\mu$  in breadth, 400 to 500  $\mu$  antero-posteriorly, and about 380  $\mu$  dorso-ventrally. Distance between suckers 350  $\mu$  or less. Pharynx about 70  $\mu$  in diameter; no prepharynx. Intestinal ceca extend almost to retractile appendage. Genital opening nearly median, close behind oral sucker. Sinus sac about 150  $\mu$  long and 110  $\mu$  broad; at its inner end a spherical prostatic vesicle, just in front of which the metraterm enters to form a distended hermaphroditic duct. Outside sinus sac vas deferens at first narrow, with prostate glands spreading wing-like on either side of it, then forming a large trilobed seminal vesicle, 280  $\mu$  long with a maximum breadth of 145  $\mu$ . Testes round or oval, situated slightly oblique, about 2/5 body length from anterior end, separated by a loop of the uterus; size from 100  $\times$  130 to 130  $\times$  200  $\mu$ . Ovary and yolk glands closely associated, widely separated from testes; ovary about 150  $\mu$  in diameter; vitelline glands, each with three or four lobes, situated on right side of body. Uterus in more or less horizontal loops behind ventral sucker, the loops extending some distance behind the vitelline glands; eggs about 18 by 11 to 12  $\mu$ .

A considerable number of species of *Sterrhurus* have been described from marine fishes by Looss, Linton, Nicoll, Manter, Stunkard and Nigrelli, Park, and



FIGS. 1 to 3: *Rhipidocotyle angusticolle* n. sp. 1, ventral view; 2, lateral view of anterior end; 3, ventral view of anterior end.

FIGS. 4 to 6: *Sterrhurus texanus* n. sp. 4, ventral view; 5, anterior end; 6, vitelline glands: upper, one gland in ventral view; lower, complex of two glands and ovary seen from right side.

Yamaguti, and there is considerable doubt as to whether all of them are valid species. Looss's (1907) type species, *musculus*, Manter's (1934) *floridensis*, and the forms assigned by Linton in 1910 to his *monticellii* (1898) are quite possibly identical, as Stunkard and Nigrelli (1934) pointed out. The species here described is a larger and much more slender form than these, with the ventral sucker much larger relative to the width, with a very short "tail," and with the ovary and vitelline gland complex situated far behind the testes, which are almost tandem in position and at some distance behind the ventral sucker. These characters, together with the blunt lobing of the vitellaria, the large tripartite seminal vesicle, and the characters of the sinus sac, distinguish this form from all members of the genus except *imocavus*, described by Looss (1907) from *Thynnus* in Egypt, which it closely resembles. It is, however, larger than *imocavus*, with less prominent dorsal lip, larger vitellaria relative to the ovary, and larger eggs.—ASA C. CHANDLER, *Rice Institute, Houston, Texas.*

A NEW SPIRUROID NEMATODE, *HABRONEMA AMERICANUM*,  
FROM THE BROAD-WINGED HAWK, *BUTEO*  
*PLATYPTERUS*

A few specimens of a *Habronema* which proved to be a new species were collected from the stomach of *Buteo platypterus* in Cherokee Co., Texas, by Mr. Rollin H. Baker, Field Biologist with the Texas Game, Fish and Oyster Commission. The specific description follows:

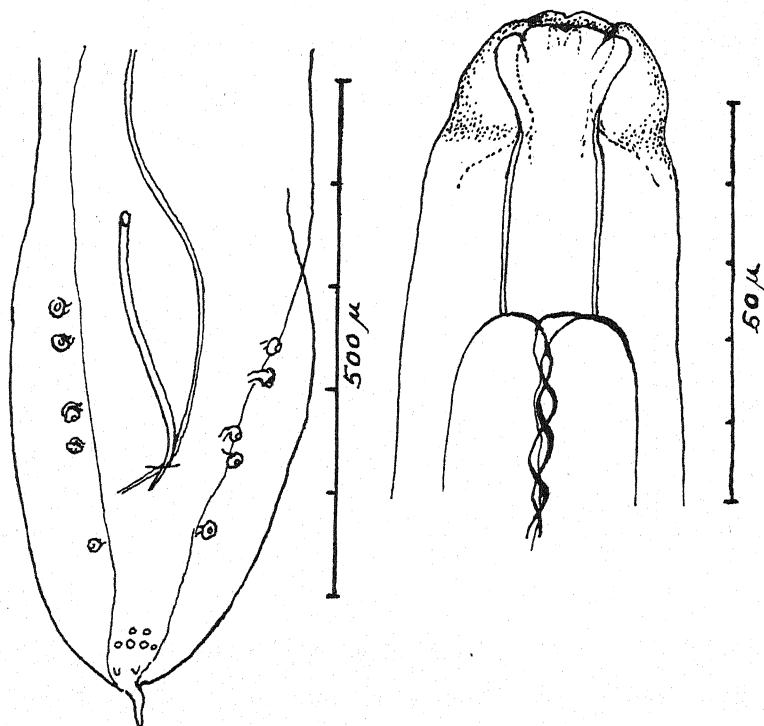


FIG. 1. *Habronema americanum* n. sp. Left, tail of male; right, head of female.

*Habronema americanum* n. sp.

Body elongate, strongly striated, with lateral alae, one better developed than the other, extending greater part of length of body. Cervical papillae anterior to nerve ring. Head indistinctly set off from body, about  $35\ \mu$  in diameter. Lateral lips broad, not very distinctly lobed; dorsal and ventral lips bluntly pointed. Bottom of oral vestibule about 38 to  $40\ \mu$  from anterior end, diameter about 13 to  $15\ \mu$ . Esophagus about 3 to 3.5 mm long.

Male about 10 mm long and  $260\ \mu$  in diameter. Caudal alae unequal, the right one longer, marked by wavy longitudinal striations. Four pairs of pedunculated preanal papillae, one similar pair postanal, and 8 very minute sessile papillae near tip of tail, arranged 2, 4, 2. Tail terminated by a fleshy spike about  $30\ \mu$  long. Spicules unequal, the right one 225 to  $250\ \mu$  long, the left one 725 to  $750\ \mu$  long; gubernaculum  $26\ \mu$  long.

Female about 15 mm long and  $350\ \mu$  in diameter. Tail conical,  $325\ \mu$  long. Vulva anterior to middle of body, dividing it about 2:3. Eggs elliptical, 37 to  $40\ \mu$  long and 18 to  $19\ \mu$  broad.

This species closely resembles *H. leptoptera* (Rudolphi, 1819) of Old World hawks, differing principally in the shape of the lips. Other minor differences are in the greater length of the esophagus, the longer spicules, and the different arrangement of the sessile papillae at the end of the tail of the male.—ASA C. CHANDLER, Rice Institute, Houston, Texas.

THE OCCURRENCE OF *HEXAMITA* (*OCTOMITUS*) *COLUMBAE* IN PIGEONS IN CALIFORNIA

Nöller and Buttgerit (Centr. Bakt. I Abt. Orig. 75: 239-240) and Buttgerit (Inaug. Dissert., Tierärstl. Hochs., Berlin) in 1923 reported a catarrhal enteritis of



young pigeons in Germany. The enteritis extended throughout the intestine and was accompanied by large numbers of a small flagellate ( $5-9\ \mu$  by  $2.5-3.5\ \mu$ ) of the genus *Hexamita* (*Octomitus*). The organisms were increasingly abundant in the lower jejunum and ileum, and most abundant 5 cm anterior to the vent. They were abundant not only in the lumen, but also deep in the crypts of the villi. There was marked hyperemia of the duodenum and jejunum, and an infiltration of lymphocytes. They named this species *Hexamita* (*Octomitus*) *columbae*.

It is the purpose of the present paper to describe four similar cases and to report *Hexamita columbae* from California. To our knowledge it is the first time this condition has been reported from pigeons in the United States.

*Case I.*—The first time that *Hexamita* was observed by us in pigeons was in a bird brought to the State Department of Agriculture Poultry Pathological Laboratory at Los Angeles. This case is referred to by Niemeyer (1939, J. Am. Vet. Med. Assn. 94: 434-435), and came from a flock which was also suffering from paratyphoid infection and trichomoniasis of the crop. The relationship of *Hexamita* to the mortality in this flock was not determined. The intestine of the one bird examined showed no marked changes. No *Hexamita* were found in the duodenum, a few were present in the jejunum and ileum, large numbers were found in the rectum and a few in the bursa of Fabricius. The absence in the upper intestine and the lack of pathology suggests that this bird was in the carrier stage rather than suffering from the presence of this parasite.

*Case II.*—The second case was a group of five birds from a flock of tumbler pigeons. Several of the young birds and one breeder in this flock were in poor condition and thought by the owner to be suffering from coccidiosis. Oöcysts were found in only one bird and then in very small numbers.

*Hexamita* was found in four of the five birds; the fifth was suffering from impaction of the rectum. Of the four birds, two were suffering from a catarrhal enteritis. The duodenum was fairly normal, but the jejunum was hyperemic and the wall of the ileum was thin. There were several diphtheritic ulcers in the rectum. *Hexamita* was absent or rare in the duodenum, numerous in the jejunum and ileum and most abundant in the rectum. It was present also in the bursa of Fabricius. No pathogenic bacteria could be isolated from these birds.

*Case III.*—The third case was brought in by the owner of the birds mentioned above. It was a banded bird which had escaped from some other loft and had been present in his loft for only a day or two. This bird was in poor flesh. There was marked catarrhal enteritis throughout the intestine and ulceration of the ileum and rectum. *Hexamita* was abundant in scrapings from the jejunum, ileum, and rectum. All of these cases originated in Southern California.

*Case IV.*—The fourth outbreak occurred in a loft located in the San Francisco Bay region. The owner reported heavy losses in his young squabs with occasional losses in the adults. A total of five pigeons of various ages were examined. All were in fair flesh, and three were suffering from a marked case of trichomoniasis of the upper digestive tract. Coccidia and a few ascarids as well as *Hexamita* were found in the intestines. The *Hexamita* were present in large numbers in the posterior two-thirds of the intestines and in small numbers in the bursae of Fabricius. In one young squab a small rectal ulcer was present and *Hexamita* in large numbers were clinging to the tissues scraped from this lesion. Because of the numbers of other parasites also present in these birds it was difficult to determine which species were responsible for the losses.

That *Hexamita columbae* is not merely a ubiquitous commensal in pigeons is evidenced by the fact that healthy pigeons have been examined from four other flocks and *Hexamita* was not present.

The disease described by Nöller and Buttgerit in Germany and also encountered in these birds in California is similar to infectious catarrhal enteritis of turkey poults (Hinshaw, McNeil, and Kofoed, 1938, Cornell Vet. 28: 281-293; McNeil, Hinshaw, and Kofoed, 1941, Am. J. Hyg. in press). One essential difference in the pathology of hexamitiasis of pigeons is the consistent catarrhal inflammation with a heavy mucus deposit in the rectum of young pigeons. This was also noted by Butt-

gereit (1923). The owner of the loft from which the pigeons of Case II came, reports that he has had only a small mortality in his young birds, but many of them are unthrifty. No other infection has been found on this ranch; so it is highly probable that *Hexamita columbae* is responsible for the loss he has had and for the stunting of the squabs.

It has not been possible to infect turkey poults with *Hexamita columbae* (cf. McNeil, Hinshaw, and Kofoed, l.c.), nor is it likely that it is possible to infect pigeons with *Hexamita meleagridis*, since the pigeons examined by us and found free from *Hexamita* were on ranches where *Hexamita meleagridis* was abundant in turkeys, and where they frequented the infected pens.—E. MCNEIL AND W. R. HINSHAW, *University of California, Division of Veterinary Science, Davis, California.*

*CERCARIA ELONGATA* BRACKETT, 1940, FROM A NEW SNAIL  
HOST, *MENETUS EXACUOUS* (SAY), IN MINNESOTA

A schistosome cercaria identified as *Cercaria elongata* Brackett, 1940 (*J. Parasitol.* 26: 195-200), was found in 2 of 40 specimens of the planorbid snail, *Menetus exacuus* (Say), collected June 22, 1940, from Lake Francis, Isanti Co., Minnesota. A small number of *Gyraulus parvus* (Say), the host Brackett reports, collected on the same date and on Sept. 9, 1940, from this lake were also positive for *C. elongata*. Although Brackett was unable to secure more than one dermatitis lesion experimentally, numerous characteristic dermatitis lesions were obtained by the writers. On one occasion when a vial containing 15 *C. elongata* from *M. exacuus* was placed in contact with the skin of the inner surface of the forearm for 12 minutes all of the cercariae penetrated and typical schistosome dermatitis lesions developed. Certainly, as Brackett concludes, this cercaria and its hosts will have to be considered in future studies of outbreaks of "swimmer's itch".—ASHTON C. CUCKLER AND LAWRENCE R. PENNER, *Department of Zoology, University of Minnesota, Minneapolis.*



JOHN EARL GUBERLET  
(1887-1940)

## IN MEMORIAM

### JOHN EARL GUBERLET (1887-1940)

John Earl Guberlet<sup>1</sup> was born at Courtland, Nebraska, March 18, 1887, the son of a farmer. He attended Bethany College at Lindsborg, Kansas, (A.B., 1909) where he studied zoology under Prof. Emil O. Deere, though for a time he was undecided as between medicine, zoology, or the ministry. The year following his graduation he spent at the University of Colorado. Here Prof. T. D. A. Cockerell interested him in the parasitology which eventually became his life-work. In 1910 Guberlet went to the University of Illinois. He worked first for his A.M. under Prof. J. S. Kingsley, his thesis being "On the Osteology of the Loricati" or rock fishes. Then he turned to parasitology for his doctorate under Prof. H. B. Ward, investigating the cestodes of poultry. He received his Ph.D. in 1914.

The year 1914-15 Guberlet was instructor in zoology at the University of Oregon, and from 1915 to 1918 professor of biology at Carroll College, Waukesha, Wisconsin. In 1918 he went to the Oklahoma Agricultural and Mechanical College at Stillwater, Oklahoma, as assistant parasitologist in the agricultural experiment station and assistant professor of zoology, being promoted in 1921 to parasitologist and full professor. In Oklahoma he went on with his parasitological investigations, specializing on the worm parasites of domestic animals.

Guberlet was called to the University of Washington at Seattle in 1923, where he was advanced to a full professorship in 1930. Beginning in 1924, he spent his summers at Friday Harbor, first on the staff of the Puget Sound Biological Station and then, after 1930, on the staff of the Oceanographic Laboratories of the University.

During these years in Seattle and at Friday Harbor, Guberlet's researches followed two main lines. He became associated with the course in fish diseases offered by the University's School of Fisheries. This led to studies on the parasites and diseases of fresh-water fishes, especially those in fish-hatcheries, and his investigations were of value in the development of pisciculture in the western portions of the state.

In connection with the marine work at Friday Harbor, he and his students<sup>2</sup> produced a notable series of studies on the flatworm parasites

<sup>1</sup> Or "Gutberlet," as he spelled it previous to 1919.

<sup>2</sup> In addition to the papers cited in the bibliography of which Guberlet was author or co-author, the following were published independently by his students:  
Folda, Florence. 1928 *Megalocotyle marginata*, a new genus of ectoparasitic trematodes from the rock fish. Publications Puget Sound Biol. Sta. 6: 195-206.  
Hart, John Francis. 1936 Cestoda from fishes of Puget Sound. II. Tetrarhynchoidea. Tr. Am. Micr. Soc. 55: 369-387.

of the marine fishes. One or two further papers on this subject were nearly ready for publication at the time of his death. He had built up a valuable parasitological collection and reprint library.

In the second place, Guberlet found the Puget Sound marine fauna new and strange. He was required to familiarize himself with it by virtue of the course in invertebrate embryology and zoology that he was teaching, and I have heard him tell how he was liberal with the midnight oil during his early sessions at the Biological Station. The result was that he came to have an extraordinarily extensive knowledge of the larger marine species. One result of this study was the book, "Animals of the Seashore," which he helped his wife, Muriel Lewin Guberlet, prepare for publication in 1936. This text, an illustrated account of nearly 200 of the common invertebrate animals of the Puget Sound region, while primarily intended for amateur and high school use, has proven to be of considerable value in the instructional work at the Oceanographic Laboratories. Another product of Guberlet's marine work was an extensive study of the distribution of the larger marine animals of Puget Sound, derived from many years of dredging mostly from the University's research M. S. *Catalyst*. Guberlet was at work on the final manuscript of this paper at the time of his death.

In addition to his instructional work in parasitology and marine zoology, Guberlet had charge of the pre-medical zoology course, and many generations of "pre-medics" have profited from his kindly sympathetic guidance. With even the largest classes he had the capacity for personalizing his relationship with his students. Those aspects of his personality which caused him at one time to consider the profession of the ministry came to the fore and made Guberlet's zoology one of the unforgettable experiences of the pre-medical student. The place he has left will be hard to fill.

As a colleague on the faculty, Guberlet was kindly and cooperative, active in the work of the faculty committees to which he belonged. He was a member of  $\Phi\Sigma\K$ ,  $\Gamma\Lambda$ ,  $\Sigma\Xi$ ,  $\Phi\K\Phi$ , and  $\Phi\Sigma$ , A.A.A.S. (fellow), American Society of Zoologists, American Microscopical Society (president, 1935), American Society of Parasitologists, American Society of Tropical Medicine, Society for Experimental Biology and Medicine, Western Society of Naturalists, and the Pacific Northwest Bird and Mammal Society (treasurer, 1930-).

During the spring of 1926 Guberlet held a visiting professorship in zoology at the University of Hawaii at Honolulu. In 1928-29 he was a delegate to the International Congress of Tropical Medicine and

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———1936 Cestoda from fishes of Puget Sound. III. Phyllobothrioidea. *Ibid* 55: 488-496.

Lloyd, Lowell Clyde. 1938 Some digenetic trematodes from Puget Sound fish. *J. Parasitol.* 24: 103-133.



Hygiene at Cairo, Egypt. At the same time he visited many of the laboratories of western and southern Europe, especially the Stazione Zoologica at Naples and the London School of Hygiene and Tropical Medicine. In 1935 he was a delegate to the 7th American Scientific Congress in Mexico City.

Early in 1937 Dr. Guberlet was stricken by a severe attack of coronary thrombosis. By June, however, he had recovered sufficiently to resume his university duties, which he continued with hardly a day's interruption for three and one-half years. Recovery, however, had only been partial. A second attack at his Seattle home shortly after five on the morning of December 30, 1940, proved fatal within a few minutes, and he died, probably without waking.

Dr. Guberlet is survived by his wife, Muriel Lewin Guberlet, whom he married on December 29, 1915, at Lindsborg, Kansas, by a son, Frank Lewin Guberlet, and by a daughter, Florence Evangeline Guberlet.—MELVILLE H. HATCH, *University of Washington, Seattle*.

THE SCIENTIFIC PUBLICATIONS OF JOHN EARL GUBERLET<sup>3</sup>

- 1915 On the osteology of some of the Loricati. *Illinois Biol. Monogr.* 2: 170-210 (5 plates, 13 Figs.).
- 1916 Morphology of adult and larval cestodes from poultry. *Tr. Am. Micr. Soc.* 35: 23-44 (30 Figs.).
- Studies on the transmission and prevention of cestode infection in chickens. *J. Am. Vet. Med. Assn.* 49: 218-237.
- 1919 On the life history of the lungworm, *Dictyocaulus filaria*, in sheep. *Ibid* 55: 621-627.
- On the life history of the chicken cestode, *Hymenolepis carioca* (Magalhaes). *J. Parasitol.* 6: 35-39 (4 Figs.).
- 1920 A new bladder fluke from the frog. *Tr. Am. Micr. Soc.* 39: 142-148 (7 Figs.).
- 1921 Studies on the sheep stomach worm—*Haemonchus contortus* Rud. *J. Am. Vet. Med. Assn.* 39: 716-721.
- Some internal parasites of Oklahoma livestock. *Oklahoma Agric. & Mech. Coll. Ext. Serv. Circ. No. 127*: 3-15.
- Stomach worms in sheep. *Oklahoma Agric. Exper. Sta. Bull. No. 137*: 3-16 (5 Figs.).
- 1922 Stomach worms in sheep. *Am. Sheep Breeder and Wool Grower* 42: 265-270.
- Some facts concerning human parasites in Oklahoma. *Oklahoma State Med. J.* 15: 187-193.
- Three new species of Holostomidae. *J. Parasitol.* 9: 6-14 (13 Figs.).
- Some notes on the parasite fauna of Oklahoma. *Proc. Oklahoma Acad. Sc.* 2: 36-41 (*Univ. Oklahoma Bull. N. S.* 247; *Univ. Stud.* 15).
- Lowery Laymon Lewis. *Science* 56: 563-564.
- Potassium nitrate poisoning in chickens. *J. Am. Vet. Med. Assn.* 62: 362-365.
- 1923 *Hemistomum confusum*, a homonym. *Tr. Am. Micr. Soc.* 42: 68.
- An epizootic of aspergillosis in chickens. *J. Am. Vet. Med. Assn.* 63: 612-620.
- Roup in poultry. *Oklahoma Agric. & Mech. Coll. Agric. Exper. Sta. Circ. July 1923*. 2 pp.

<sup>3</sup> The first three titles were published under the name of "Gutberlet."

- Parasites of dogs and cats of Oklahoma. *Proc. Oklahoma Acad. Sc.* 3: 71-78 (Univ. Oklahoma Bull. N. S. 271; Univ. Stud. 16).
- 1924 Notes on the life history of *Ascaridia perspicillum* (Rud.). *Tr. Am. Micr. Soc.* 43: 152-156.
- 1925 *Malacobdella grossa* from the Pacific coast of North America. *Publications Puget Sound Biol. Sta.* 5: 1-13 (8 Figs.).
- 1926 Ecto-parasitic infusoria attacking fish of the Northwest. *Univ. Washington Publications Fish.* 2: 1-16 (5 Figs.).
- Notes on the parasitic fauna of Hawaii. *Proc. Hawaiian Acad. Sc., First Ann. Meeting*, Bernice P. Bishop Mus. Spec. Publ. 11: 29-30.
- 1927 Some relationships of the parasitic flatworms of the birds of the Northwest. *Murrelet* 8: 1-3 (mimeographed).
- (with Harry A. Hansen and Jean A. Kavanagh) Studies on the control of *Gyrodactylus*. *Univ. Washington Publications Fish.* 2: 17-29.
- 1928 Parasitic worms of Hawaiian chickens with a description of a new trematode. *Tr. Am. Micr. Soc.* 47: 444-453 (5 Figs.).
- Notes on a species of *Argulus* from goldfish. *Univ. Washington Publications Fish.* 2: 31-41 (7 Figs.).
- Observations on the spawning habits of *Melibe leonina* (Gould). *Publications Puget Sound Biol. Sta.* 6: 263-270.
- Two new genera of trematodes from a red-bellied water snake. *J. Helm.* 6: 205-218 (13 Figs.).
- 1930 Notes on relationships of parasitic flatworms to birds and mammals. *Murrelet* 11: 15-17.
- 1931 (with V. S. Samson and W. H. Brown.) A report of hydrocoele embryonalis, or yolk-sac disease, in salmon fry, with a note on its cause. *Tr. Am. Micr. Soc.* 50: 164-167.
- 1932 Parasitism in Northwestern United States of North America in relationship to public health. *Compt. Rend. Cong. Internat. Méd. Trop. et Hyg. (Le Caire, Déc. 1928)* 4: 41-47.
- (with Lowell C. Lloyd) A new genus and species of Monorchidae. *J. Parasitol.* 18: 232-239 (5 Figs.).
- Notes on some Onchocotylineae from Naples with a description of a new species. *Pubblicazioni Stazione Zool. Napoli* 12: 323-336 (10 Figs.).
- 1933 (with R. C. Miller) Notes on birds observed at sea off the North Pacific coast. *Murrelet* 14: 7-8 (for correction see *Ibid* 14: 46).
- 1934 Recent advances in our knowledge of the parasites of the marine fishes of the Pacific. *Proc. 5 Pacific Sc. Cong. (Canada 1933)*, pp. 4165-4169.
- Observations on the spawning and development of some Pacific annelids. *Ibid*, pp. 4213-4220.
- 1936 (with Lowell C. Lloyd) *Syncoclium filiferum* (Sars) from the Pacific salmon. *Tr. Am. Micr. Soc.* 55: 44-48 (5 Figs.).
- (with John F. Hart) Cestoda from fishes of Puget Sound. I. Spathebothriidea, a new superfamily. *Ibid* 55: 199-207 (13 Figs.).
- A brief résumé of trematode studies in Washington. *The Biolog (Univ. Portland)* 3: 1-2; 9-10.
- Trematodos ectoparasitos de los peces de las costas del Pacifico. *An. Inst. Biol., Univ. Nac. Mexico* 7: 457-467 (7 Figs.).
- Note on embryo porpoise. *Murrelet* 17: 56.
- Two new ectoparasitic trematodes from the sting ray, *Myliobatus californicus*. *Am. Midland Naturalist* 17: 954-964 (12 Figs.).
- 1937 Lowell Clyde Lloyd (1903-1936). *Tr. Am. Micr. Soc.* 56: 119.
- (with Kelshaw Bonham) Notes on *Microcotyle sebastis* Goto from Puget Sound. *J. Parasitol.* 23: 281-290 (14 Figs.).
- A method for rearing *Nereis agassizi* and *N. procera*. In Galstoff et al: *Culture Methods for Invertebrate Animals*, Ithaca, N. Y., Comstock Pub. Co., pp. 184-185.

- 1938 (with Kelshaw Bonham) Ectoparasitic trematodes of Puget Sound fishes—  
Acanthocotyle. Am. Midland Naturalist 20: 590-602 (10 Figs.).  
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1940 (with H. H. Hotson) A fly maggot attacking young birds, with observations  
on its life history. Ibid 21: 65-68 (4 Figs.).

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# The Journal of Parasitology

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## CHARLES WARDELL STILES 1867-1941

The death on January 24, 1941, of Dr. Charles Wardell Stiles, retired medical director of the United States Public Health Service and for 30 years chief of the Division of Zoology of the National Institute of Health (formerly the Hygienic Laboratory), marked the passing of a pioneer American zoologist and virtually the end of an epoch in American parasitology.\*

Dr. Stiles was born at Spring Valley, N. Y., on May 15, 1867, the son of Rev. Samuel Martin and Elizabeth White Stiles. Part of his undergraduate work was taken at Wesleyan University but in 1886 he enrolled in the Collège de France and later attended the University of Berlin. He obtained his A.M. and Ph.D. degrees at Leipzig. The year 1891 found him at the Trieste Zoological Station and later at the Pasteur Institute. On his return to the United States later in that year he was appointed to the position of Zoologist in the United States Bureau of Animal Industry, where, along with Curtice and Hassall, he laid the foundations for the zoological work of that Bureau and participated in the outstanding achievements which marked its progress.

One of Dr. Stiles' first assignments in the Bureau of Animal Industry was to compile a report on the animal parasites of cattle. In preparing the material on cestodes, so many contradictions in the diagnoses of species were encountered that he found it necessary to restudy the whole group, a study which resulted in the publication in 1893 by Stiles and Hassall of the bulletin entitled "A revision of the adult cestodes of cattle, sheep and allied animals." It has been said that Stiles evinced little interest in the subject of zoological nomenclature until his election to the secretaryship of the International Commission. However, in his first major publication he discussed at some length the question of nomenclature in relation to his problem and observed that "the only way to establish an international nomenclature in medical zoology, as well as in other branches of biology, is to enforce the law of priority." The foresight and soundness of the

\* An excellent portrait of Dr. Stiles was published in this JOURNAL in June, 1933 (19: 257), accompanying an article by Benjamin Schwartz entitled "A Brief Resume of Dr. Stiles' Contribution to Parasitology." In the August, 1939, issue appeared Dr. Stiles' reminiscent "Early History, in Part Esoteric, of the Hookworm (Uncinariasis) Campaign in our Southern United States."

work in this bulletin are exemplified by some of the conclusions summarized, as follows: (1) Descriptions of cestodes based upon external form alone, unassociated with internal anatomy, are of little value. (2) The present genus *Taenia*, as generally accepted by authors, contains forms which must be restudied and arranged in several sub-families and a number of genera. (3) This revision must be based on internal anatomy. (4) In the adult cestodes of cattle and sheep three genera, *Moniezia*, R. Bl., *Thysanosoma* Dies., and *Stilesia* Rail., are recognized. In this publication, Stiles reported briefly on life history studies with *Moniezia expansa*. While the experiments with the feeding of ova to sheep and the attempts to infect various invertebrate hosts were entirely negative, Stiles predicted that "some insect, worm, or snail will be found to contain the larval stage."

Stiles' bulletin on tapeworms of poultry in 1896 laid down some very specific criteria for cestode life history studies. Suggested methods of investigation were cited, as follows: (1) Experimental infection by feeding known larval stages in invertebrates; (2) experimental infection of invertebrates by feeding ova of bird tapeworms; (3) comparison of hooks on adults with hooks on larval stages; and (4) wild speculation totally devoid of any scientific foundation. Stiles commented: "The less said about the fourth method the better."

Stiles' continued interest in the cestodes was further reflected by the publication in 1896 of "A revision of the adult tapeworms of hares and rabbits." In this bulletin it was noted that none of the adult leporine tapeworms described from Europe had been found in America and that American forms described as *Taenia pectinata* must be distributed over several species. *Anoplocephala*, *Cittotaenia* and *Davainea* were considered as valid genera with the impression that *Andrya* and *Bertia* would be recognized as valid when sufficient material became available for study.

In his report on the verminous diseases of cattle, sheep and goats in Texas, Dr. Stiles made some very practical contributions to the subject of ruminant parasites. His critical anthelmintic tests were among the first to be conducted on ruminants and the measures which he advocated for the control of strongylid parasites were particularly sound when viewed in the light of our broader present day knowledge. The inefficacy of intratracheal injections for the treatment of lungworm infections was noted; and Stiles in this investigation was apparently the first to carry out experiments on the mechanism of the passage of fluids to the ruminant stomach.

During the years 1898 and 1899, Dr. Stiles served as agricultural attaché to the American Embassy in Berlin and conducted investigations to determine the value of the microscopic inspection of pork for trichinae, to ascertain whether any cases of trichinosis could be traced to American

pork inspected by the United States Department of Agriculture and to investigate the charges brought in Germany against American meats. It will be recalled that in 1880 Germany prohibited the importation of sausage and chopped pork products from the United States and in 1883 excluded all American pork. By the decree of 1891, following the Saratoga Convention, American pork products were readmitted with the understanding that the United States Department of Agriculture should inspect them microscopically for trichinae before shipment. Microscopic inspection was then instituted. However, while the Imperial German Government recognized the certificates of inspection as valid for customs purposes, no agreement was made by which any kingdom, state or local authority was required to recognize the documents for sanitary or health purposes. As a consequence, local restrictions were promulgated which interfered seriously with the flow of American pork into the channels of German trade.

Dr. Stiles' exhaustive investigations and his subsequent voluminous report showed in substance that not a single case of trichinosis in Germany had ever been traced to American certified pork, that claims for the causation of trichinosis by uncertified American pork were either unjustified or could not be substantiated by the evidence, and that the microscopic inspection of pork in Germany was a very costly procedure and led to a false sense of security since cases of the disease were known to have occurred following the ingestion of such pork.

While Dr. Stiles' contributions to veterinary parasitology were of outstanding merit, this earlier work has been overshadowed by his achievements in the field of public health and it is for the latter that he will be most remembered. As a preliminary excursion into this field, while still in the Bureau of Animal Industry, he compiled at the request of Surgeon General Walter Wyman of the United States Public Health Service a report on "The significance of the recent American cases of hookworm disease (uncinariasis, or anchylostomiasis) in man," and a few months later on May 10, 1902, he described for the first time the New World hookworm, *Uncinaria americana*, later renamed by him *Necator americanus*. In his lectures to medical students, Stiles had frequently expressed the view that hookworm disease probably existed in the southern United States, and a statement in the paper in question crystallized this view in the following words:

"*Uncinaria americana* is known to occur in Texas, in Virginia, and in Puerto Rico, and this wide geographic separation shows very clearly that in the new world we have a special, heretofore undescribed parasite which causes uncinariasis. This further indicates very strongly the correctness of the view that uncinariasis is endemic in the southern states, although it is rarely recognized."

On August 16, 1902, Dr. Stiles was appointed Professor of Zoology in the United States Public Health Service and organized the newly

authorized Division of Zoology in the Hygienic Laboratory. Shortly thereafter, he set out on an investigational tour of the South and on October 24, 1902, published a preliminary report of his findings which indicated in substance that hookworm disease was one of the most important and most common diseases of the South. The report concluded in part with the following prophetic words:

"All of the cases thus far examined are due to *Uncinaria americana*, demonstrating clearly that this is an endemic infection. . . . There is, in fact, not the slightest room for doubt that uncinariasis is one of the most important and most common diseases of this part of the South, especially on farms and plantations in sandy districts, and indications are not entirely lacking that much of the trouble popularly attributed to 'dirt-eating,' 'resin-chewing,' and even some of the proverbial laziness of the poorer classes of the white population are in reality various manifestations of uncinariasis."

The soundness of this view became increasingly evident as other investigators, notably Dr. H. F. Harris of the Georgia State Board of Health, added confirmation to the findings but it was Dr. Stiles who inspired and motivated the hookworm campaign which followed.

In his contemporary history "Our Times," Mark Sullivan relates very entertainingly how hookworm disease came to be brought forcibly to the attention of the American people. Dr. Stiles had been detailed by Surgeon General Walter Wyman to present before the Pan-American Sanitary Conference held in Washington, D. C., December 4, 1902, a report of his investigational field trip in the South. As the paper was being read, the polysyllabic words seemed to act as a somnifacient on the apathetic cerebrum of a young man slumped in a seat in the front row of the hall. Suddenly, as Dr. Stiles brought out his point that hookworm disease might be the cause of the so-called laziness of the South, the young man's mental faculties sprang to life again and his flying fingers spread penciled words across the pages of a notebook which had almost fallen from his inert hand. The strange reawakening of the young man remained a mystery to Dr. Stiles until the next day when the staring headlines of the New York Sun informed him that he had discovered the "Germ of Laziness."

While the facetious headline and the humorous story carried under its banner created considerable controversy and engendered harsh criticism, particularly in the South, the article no doubt was responsible more than any other one thing for bringing to the attention of the medical profession and the public in general the importance of hookworm disease.

In the face of the criticism which arose like an enveloping cloud, Dr. Stiles carried the hookworm message to the South and persistently pursued an educational campaign designed to convince the medical profession, public health officials and the hookworm victim himself of the seriousness of the situation. He wrote papers, articles and bulletins; lectured before medical groups and other audiences; promoted the es-



tablishment of hookworm clinics; organized hookworm instruction courses in the schools; and did a prodigious amount of spade work in preparing the South for the control campaign which was to follow.

The Act of January 29, 1907, providing for an investigation of woman and child labor in the United States, gave further opportunity for dealing with the problem. Dr. Stiles was detailed to investigate health conditions among cotton mill workers. Studies conducted in 169 mills and establishments in 6 Southern States and 26 mills in 3 New England States disclosed the fact that the so-called "cotton mill anemia" of the South, which had been assumed to be due to the inhalation of cotton lint, was in reality a manifestation of hookworm disease.

In 1908 and 1909, Dr. Stiles cooperated closely with the Country Life Commission appointed by President Theodore Roosevelt and assumed a prominent part in extensive studies in rural sanitation. As an outgrowth of this and Dr. Stiles' other work, Mr. John D. Rockefeller became interested in the problem of hookworm disease, an interest which culminated in the formation in 1909 of the Rockefeller Sanitary Commission and a gift of \$1,000,000 to finance a five-year campaign against the disease. Dr. Stiles became a member of the Commission and was made its scientific secretary, a post which he retained throughout the life of the organization.

The discovery of a new species of hookworm widespread in America, the vigorous campaign for recognition of the public health importance of the parasite and its culmination in the application of extensive control work probably mark the high lights of Dr. Stiles' career. However, during the intervening years he was not idle in other fields. In the meanwhile his report on surra and his "Animal parasites of cattle" had appeared as Bureau of Animal Industry publications. His "Illustrated key to the trematode parasites of man" appeared in 1904, to be followed in 1906 by a similar key to the cestodes of man. In 1905, he conducted an investigation into the cause, transmission and source of Rocky Mountain spotted fever. While Dr. Stiles was unable to confirm the hypothesis that this disease is transmitted by ticks, he did disapprove the contention of Wilson and Chowning that it was caused by a *Piroplasma*, the so-called *Piroplasma hominis*. His later valuable contribution on "The taxonomic value of the microscopic structure of the stigmal plates in the tick genus *Dermacentor*" was probably an outgrowth of his spotted fever investigations.

In 1910, there appeared the monograph by Stiles and Goldberger on an anatomical study of *Watsonius watsoni* and 19 allied species of the superfamily, Paramphistomoidea. This outstanding work represented a very valuable contribution to the internal anatomy of a group requiring prolonged and detailed study.

During the years between 1902 and 1912, Dr. Stiles collaborated with

Dr. Albert Hassall in the preparation of the monumental Index Catalogue of Medical and Veterinary Zoology, which was issued in 36 parts. In addition Dr. Stiles and his associates later prepared and published several subject catalogues which appeared as Hygienic Laboratory Bulletins, as did a number of key-catalogues of various parasites reported from man and other animals. These catalogues constituted a summary of the world's literature on zooparasitic infections, and as such are familiar to every parasitologist.

Stiles had very early in the hookworm work recognized the importance of environmental sanitation and from the beginning the control campaigns which he directed were predicated on the thesis of "80 percent prevention and 20 percent treatment." Naturally, he became involved more and more in the sanitation problem. For instance during his work with the Country Life Commission, he inspected 4,645 farm homes in 6 states and found 55.2 per cent of them without toilet facilities of any sort. These activities led later to intensive rural sanitation campaigns by officers of the United States Public Health Service in cooperation with the State boards of health, during which from 1914 to 1917, a total of 105,157 rural homes, 731 churches and 961 rural schools were inspected for sanitary facilities.

In 1910, Stiles began studies on soil pollution and the survival of parasite cysts and ova under varying conditions. These investigations were interrupted by the first World War but after the war they were resumed and pursued intensively. Of all of Dr. Stiles' work, these studies are probably the least known to parasitologists. However, very important findings came from them and one eminent authority in the field has stated only recently: "The results of ground water pollution studies by Stiles and Crohurst established accurately and for all time the rate and level at which fecal pollution spreads in a medium composed of sand having a subsurface ground-water table."

Following the armistice marking the end of the first World War, some apprehension was felt on the part of public health authorities that soldiers returning from overseas service might serve as unusual sources of infection for the dissemination of *Endamoeba histolytica* and other intestinal parasites. In a survey involving over 8,000 persons, including returned soldiers, soldiers who had not served overseas, civilians, and immigrants, Boeck and Stiles determined that individuals of the first mentioned group were no more frequently infected with *E. histolytica* and other intestinal parasites on the average than were individuals comprising the other groups and that therefore members of the A. E. F. could not be expected to serve as unusual sources of infection in the dissemination of parasitic diseases.

Dr. Stiles was elected secretary of the International Commission on

Zoological Nomenclature at the International Zoological Congress at Cambridge in 1898 and until 1936 served faithfully and untiringly in exerting an influence which had much to do with bringing about proper usage of zoological terminology. He represented the United States at the international congresses at Leyden, 1895, Cambridge, 1898, Berlin, 1901, Berne, 1904, Boston, 1907, Gratz, 1910, Monaco, 1913, Budapest, 1927 and Padua, 1930.

During his long and distinguished career, many honors came to Dr. Stiles. He received honorary M.S. and D.Sc. degrees from Wesleyan University, LL.D. from the University of North Carolina, D.Sc. from Yale and M.D. from the Richmond Medical College. He was a foreign correspondent of the Société de Biologie of France and of the Académie de Médecine of France, a corresponding member of the Zoological Society of London, and an honorary member of other foreign societies.

Dr. Stiles' talents were well illustrated by the scope of his researches and his broad, ever changing horizons. In the light of present day specialization, his versatility was amazing. It enabled him to produce important contributions on nematodes, trematodes, cestodes and arthropods, to achieve conspicuous leadership in the field of zoological nomenclature, to prepare with infinite care and precision outstanding catalogues of incalculable value for all time, and to draft, motivate and participate actively in broad public health programs. Perhaps of all his scientific accomplishments, his influence in the field of public health was most far reaching and yet most undervalued. In one of his later papers published shortly before his retirement from the Public Health Service, Stiles enumerated ten factors responsible for the marked advancement in public health in the South. Very generously he omitted reference to the influence of his own work in bringing about this advancement. Today the Southern States may well point with pride to their exceptional rural health organizations. There is no doubt but that these organizations had their foundations in the early hookworm work and that the health consciousness of the South was awakened by the message which Dr. Stiles carried into its highways and byways at the turn of the century.—WILLARD H. WRIGHT, *Division of Zoology, National Institute of Health*.



# THE OÖCYSTS OF COCCIDIA FROM DOMESTIC CATTLE IN ALABAMA (U. S. A.), WITH DESCRIPTIONS OF TWO NEW SPECIES

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There is little information available on the bovine coccidia to supplement oöcyst studies in the definition of species. The relative pathogenicity, localization in the tissues, and morphology of the intracellular stages of coccidia from bovines have not been worked out on a comparative basis. To be reliable, such information should be based on experimental work with pure cultures and coccidia-free hosts. Considering the difficulties involved in providing these experimental conditions with bovines, a long time will elapse before speciation in these coccidia can be established with a thoroughness approaching that accomplished for chicken coccidia.

The deficiencies in our knowledge of complete life histories and the probable extended period before such information will be available in considerable volume make all the more desirable at the present time a system of diagnosis based on comparative morphology of oöcysts. The present survey of coccidial oöcysts from domestic cattle in the vicinity of the U. S. Regional Animal Disease Research Laboratory at Auburn, Alabama, where this study was made, was carried out to determine whether the groups of oöcysts previously described as species constitute recognizable entities from host to host and, in the event this were true, to determine the species prevalent in bovines from that section of the country. The study revealed 9 distinct groups of oöcysts which were differentiated by morphological and physiological criteria, 7 of these fitting the definitions for described species from bovines while 2 are apparently new. This paper presents comparative descriptions of these oöcysts which, it is hoped, will contribute toward more accurate designations of coccidial infections in bovines under field and experimental conditions.

## MATERIALS AND METHODS

Oöcysts for this study were obtained from fecal specimens from calves having natural coccidial infections. Most of the host animals were male and female Jersey calves and a few of the Hereford breed. The entire early natural infection was followed by frequent fecal examinations in 7 healthy calves kept on the premises of the Laboratory. Fecal samples

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were obtained at intervals during August and September, 1938, from 25 unthrifty calves from a dairy herd in the vicinity, most of these animals having shown more or less extensive scouring and emaciation during the second to fifth months of life and occasionally passing diarrhetic stools containing blood. Individual specimens were obtained from several host animals from different localities in central Alabama. The calves in which natural infections were followed by frequent examinations provided the best source of material, since each calf after beginning to discharge oöcysts at the age of 4 to 6 weeks remained a continuous source of supply for the following several weeks, and usually discharged oöcysts of most species of bovine coccidia before the initial heavy natural infection subsided.

In the laboratory, flotations were made by mixing small amounts of strained fecal sediment with 15 volumes of 40 per cent sugar solution. After flotation, oöcysts in the surface films were removed with a wire loop 0.6 cm in diameter for observation, measurements and sporulation tests. Photographs, observations and drawings were made on oöcysts in temporary mounts in the flotation fluid, which had satisfactory optical qualities for observation and was ideal for temporary mounts because of high viscosity and slow evaporation. Measurements were made on oöcysts in hanging drops of flotation fluid on 22 mm coverslips inverted over the pits of depression slides. Secondary flotation occurred and oöcysts soon accumulated beneath the surfaces of the coverslips, orienting themselves with their long axes parallel to the surface of the glass. Individual oöcysts were then measured on the scale of an ocular micrometer, and the resulting readings transposed into microns. This method of measuring concentrated the oöcysts into a relatively small space when specimens in a sample were few, and removed the danger of distortion through coverslip pressure. When observations and measurements were made within a few hours after flotation, the sugar solution had no perceptible effect on shape, size or color of oöcysts.

Sporulation times for oöcysts were determined by a refinement of the method described by the writer (1938) for obtaining standard conditions for sporulation of oöcysts of sheep coccidia. Loopfuls of flotation fluid containing freshly discharged oöcysts were placed on 22 mm coverslips and each drop encircled with a strong line made by a wax pencil. Two drops of clean tap water were added to each drop of fluid on a coverslip and the resulting larger drop thoroughly mixed to insure dilution of the sugar solution and allow the oöcysts to sink through the fluid to the surface of the glass. The circle made by the wax pencil served to keep the drop intact. Preparations were made only from loopfuls of flotation material containing little fecal debris, since it was desired to eliminate putrefaction and, therefore, standardize the oxygen supply.

These coverslip preparations were then placed upright on inverted dishes inside a closed Petri dish containing a shallow layer of water to maintain humidity and prevent evaporation from the coverslip drops. Observations for sporulation were made at 24-hour intervals in the following manner: Excess water was removed from each drop by applying the tip of a piece of blotting paper to the edge, care being taken to leave a shallow layer over the oöcysts adhering to the surface of the coverslip. The coverslip was then inverted over the pit of a depression slide, the oöcysts remaining attached to the surface of the glass. Observations were usually made under the high dry objective, but it was possible to use oil immersion when small oöcysts were being observed. At each observation the progress of sporulation was tabulated. Until the sporulation process was complete in oöcysts which were being tested coverslips were returned to the moist chamber for another 24-hour period, care being taken to restore the two drops of water to each preparation. Since all tests were performed at room temperature and aeration and moisture standardized by maintaining similar amounts of clean water in all preparations, the results of sporulation tests became comparative and proved to be a valuable accessory criterion for the identification of certain oöcysts.

#### DESCRIPTION OF SPECIES

*Eimeria zurnii* (Rivolta, 1878) Martin, 1909  
(Plate I, 2; Plate II, 12)

*Synonyms:* *Cytospermium zurnii* Rivolta, 1878; *Coccidium bovis* Züblin, 1908, pro parte; *Eimeria bovis* (Züblin, 1908) Fiebiger, 1912, pro parte; *Eimeria canadensis* Bruce, 1921, pro parte.

*Unsporulated oöcysts:* Oöcysts of *E. zurnii* from Alabama calves measured 15 to 22 (average 17.8)  $\mu$  long by 13 to 18 (average 15.6)  $\mu$  in transdiameter; 20 to 40 consecutive oöcysts were measured from each of 5 host animals, dimensions being based on a total of 140. Shape spherical to bluntly ellipsoidal; oöcysts 0.73 to 1.00 (average 0.88) as broad as long. No micropyle visible. Wall thin, homogeneous, transparent, of uniform thickness throughout; the oöcysts appear colorless under low magnification, but under oil immersion the wall shows a faint grayish-lavender tint, fading to light yellow at one end. In pure discharges these oöcysts comprise a uniform, well-defined group.

*Sporulation:* In 4 trials at room temperature 76 to 100 per cent of the oöcysts sporulated completely within 48 to 72 hours after isolation into a drop of clean tap water.

*Relationships:* The more ellipsoidal oöcysts of *E. zurnii* may be confused with those of *E. ellipsoidalis*, which are usually found in large numbers in feces from healthy calves rather than in diarrheic feces from calves with clinical coccidiosis. The smaller subspherical oöcysts of the species resemble those of *E. subspherica* morphologically, but they sporulate faster, have sturdier walls and are usually associated with clinical coccidiosis.

*Occurrence:* Oöcysts of *E. zurnii* are often associated in pure or nearly pure infection with bloody, diarrheic feces or bloody mucus from calves, indicating that this species is probably highly pathogenic. In the present study large numbers of oöcysts of *E. zurnii* were found in pure infection in scouring calves, once in bloody mucus and once in slightly bloody, diarrheic feces; in another bloody, diarrheic

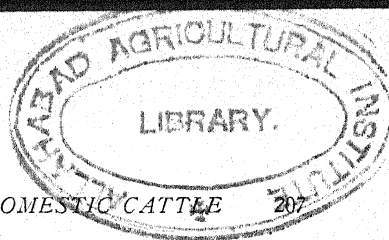
stool and in a diarrheic stool with no blood, oöcysts of *E. zurnii* comprised at least 95 per cent of the total oöcyst count, while oöcysts of *E. bovis* constituted the remainder.

Although others had indicated that there were at least two types of coccidial oöcysts to be found in feces from domestic cattle, it remained for Yakimov and Galouzo (1927) to separate the ovoidal oöcysts mentioned by the earlier investigators from those described as spherical. They retained the name *E. zurnii* for the spherical oöcysts and gave the dimensions as  $17.1\ \mu$  in diameter, with a range of  $15.3$  to  $19.1\ \mu$ . The larger, ovoidal oöcysts were described by these writers as *E. smithi*. While the oöcysts of *E. zurnii* found in diarrheic calves from Alabama were similar in size to those measured by Yakimov and Galouzo, the usual oöcyst was found to be subspherical rather than regularly spherical, with a range in shape from spherical to bluntly ellipsoidal.

Rivolta (1878) did not give sufficient information to enable later investigators to determine accurately which species of bovine coccidia he meant in his description of *Cytospermium zurnii*, later properly referred to the genus *Eimeria*. Since he was dealing with coccidia associated with bloody dysentery in young cattle, it has been assumed that he was describing the coccidium represented by the spherical and subspherical oöcysts frequently associated with bloody diarrhea in calves and later specified for *E. zurnii* by Yakimov and Galouzo. Rivolta is therefore credited with authorship of the species.

In his *Coccidium bovis*, Züblin (1908) described oöcysts of at least 2 species, including those fitting the description of *E. zurnii*. Most of the coccidia from bovines were said by this writer to be "runde oder ovale." and to have a "Durchmesser von  $0,012 : 0,010$  Millimeter bis  $0,025 : 0,020$  mm." While these dimensions have sufficient range to fit oöcysts of several species of cattle coccidia, Züblin's statement that rounded or spherical ("kugelig") forms predominated in numbers and the fact that his observations were made on animals with clinical coccidiosis make it reasonably certain that his *E. bovis* included oöcysts defined as *E. zurnii*. It appears, therefore, that Becker (1934) was justified in synonymizing *E. bovis* in part with *E. zurnii*.

Bruce (1921) described *E. canadensis* from coccidia from cattle in British Columbia. The oöcysts were said to be spherical, ellipsoidal and ovoidal in shape and to vary from  $11.6$  to  $43.1\ \mu$  in length and from  $11.6$  to  $27.8\ \mu$  in breadth. His figures included several bluntly ellipsoidal to spherical oöcysts measuring  $16.6$  to  $18.2\ \mu$  by  $13.2$  to  $17.4\ \mu$ , dimensions which fall within the range for *E. zurnii*. Since there is little doubt that Bruce included *E. zurnii* in his species, Becker (1934) was justified in synonymizing the name *E. canadensis* in part with the earlier species.



CHRISTENSEN—COCCIDIA FROM DOMESTIC CATTLE 207

*Eimeria bovis* (Züblin, 1908) Fiebiger, 1912  
(Plate I, 7; Plate II, 17)

*Synonyms:* *Coccidium bovis* Züblin, 1908, pro parte; *Eimeria canadensis* Bruce, 1921, pro parte; *Eimeria smithi* Yakimov and Galouzo, 1927.

*Unsporulated oöcysts:* Oöcysts of this species from Alabama calves measured 23 to 34 (average 27.7)  $\mu$  long by 17 to 23 (average 20.3)  $\mu$  in transdiameter; 10 to 40 consecutive oöcysts were measured from each of 25 fecal specimens from 8 host animals, dimensions being based on a total of 500. Shape typically stout ovoidal, but somewhat blunted across narrow end; variation considerable, especially in heavy discharges, with subellipsoidal, asymmetrical and elongated, sharply tapered oöcysts occurring at extremes; 0.56 to 0.88 (average 0.73) as broad as long. Micropyle present as a gap in wall at tapered end of oöcyst, but perceived as a lightened area rather than as a definite opening. Wall homogeneous, transparent and slightly thinner toward micropylar end. The pale, cloudy, greenish- to yellowish-brown color of these oöcysts under low magnification is intermediate between that of the relatively colorless oöcysts of *E. zurnii* and the distinctly yellowish-brown oöcysts of *E. auburnensis*.

*Sporulation:* In 10 trials at room temperature 63 to 100 per cent of the oöcysts of *E. bovis* sporulated completely within 48 to 72 hours after isolation into a drop of clean tap water.

*Relationships:* The general shape of typical oöcysts of *E. bovis* is similar to that for *E. alabamensis*, but oöcysts of *E. bovis* are considerably larger, sporulate faster, and do not have such delicate walls. Oöcysts of *E. bovis* are significantly smaller than those of *E. auburnensis* or *E. bukidnonensis*, which are also tapered toward one end; the wall shows no tendency toward a roughened phase as in *E. auburnensis* and is not thickened as in *E. bukidnonensis*. In color, oöcysts of *E. bovis* are about intermediate between the relatively colorless *E. alabamensis* and the distinctly yellowish-brown *E. auburnensis*.

*Occurrence:* Oöcysts of *E. bovis* were frequently found in large numbers in feces from healthy calves with natural infections. They were occasionally found associated with those of *E. zurnii* in stools from emaciated, scouring calves.

In describing coccidia associated with bloody dysentery in cattle, Züblin (1908) stated that occasionally "betrug die Breite 0,020 mm, die Länge 0,030–0,035 mm," although predominantly they were said to be "runde oder ovale" and to measure "0,012 : 0,010 Millimeter bis 0,025 : 0,020 mm." Comparing the bovine coccidia with those known from the rabbit, Züblin added that the forms from cattle were almost exclusively spherical ("kugelig"), rarely ellipsoidal ("länglich rund"), while occasionally larger, egg-shaped specimens ("grössere, eiförmige Exemplare") were found. Züblin concluded in his summary that the coccidium from cattle, which he named *Coccidium bovis*, was "rundlich" and measured 0.015 mm in length by 0.012 mm in breadth. No mention was made here of the "ovale" or "grössere, eiförmige" oöcysts described in the body of the paper, but since Züblin did not distinguish more than one coccidium from cattle the presumption is that he included these forms in his *E. bovis*.

Since Züblin was dealing with coccidia from animals with coccidial dysentery, it seems to be a safe conclusion that the oöcysts he described as "rundlich" or "kugelig" were those later defined for *E. zurnii*. On the basis of size only, his relatively rare, larger oöcysts measuring 30 to

35  $\mu$  in length by 20  $\mu$  in transdiameter might be included with narrower oöcysts of *E. canadensis* or with larger oöcysts of the coccidium described as *E. smithi* by Yakimov and Galouzo (1927) from ovoidal oöcysts measuring 25.2 to 32.4 (average 31.5)  $\mu$  in length by 19.8 to 28.8 (average 21.6)  $\mu$  in transdiameter. However, Züblin's additional statement that occasionally "grössere, eiförmige" specimens occurred creates the strong impression that this description applied to the oöcysts measuring 30 to 35  $\mu$  by 20  $\mu$ . If this is true, these oöcysts fit the description of the ovoidal *E. smithi* rather than the ellipsoidal *E. canadensis*. Becker (1934) evidently considered that the original description of *E. bovis* included oöcysts later described for *E. smithi*, since he synonymized the former in part with *E. smithi*, which he designated as the valid name for the species.

While there may be some doubt as to whether Züblin's oöcysts measuring 30 to 35  $\mu$  in length by 20  $\mu$  in breadth are those he described as "grössere, eiförmige Exemplare," the inference that these descriptions apply to the same oöcysts seems to be strong enough to warrant the opinion that part of this author's *E. bovis* represents the coccidium later described as *E. smithi*. The present writer feels that the name *E. bovis* should be retained because part of the description of this species defines with reasonable accuracy oöcysts later described for *E. smithi*. Under this opinion, the name *E. bovis* is restricted to apply only to the oöcysts in question, and the name *E. smithi* is reduced to synonymy.

*Eimeria canadensis* Bruce, 1921  
(Plate I, 8; Plate II, 19, 20)

*Synonym: Eimeria zurnabadensis* Yakimov, 1931.

*Unsporulated oöcysts:* Oöcysts answering the description for this species from Alabama calves measured 28 to 37 (average 32.5)  $\mu$  long by 20 to 27 (average 23.4)  $\mu$  in transdiameter; 5 to 30 oöcysts were measured from each of 10 fecal samples from 8 host animals, dimensions being based on a total of 170. Shape typically regularly ellipsoidal, varying from nearly cylindrical to stoutly ellipsoidal, with a few specimens being slightly tapered; 0.62 to 0.85 (average 0.72) as broad as long. Micropyle an inconspicuous gap in wall at one end, appearing covered with a thin, dark refraction line which possibly represents an operculum. Wall transparent, about 1  $\mu$  in thickness in main body of oöcyst, slightly thinner at each end; impregnated with brown, fading to paler yellowish-brown toward ends, imparting a delicate tint to oöcyst. Oöcyst membrane distinguishable from inner surface of wall, the two not forming the single, heavy refraction line characteristic of smaller oöcysts. These are relatively large, stoutly ellipsoidal, delicately yellowish-brown oöcysts.

*Sporulation:* In 6 trials at room temperature 66 to 100 per cent of the oöcysts observed required 72 to 96 hours for complete sporulation after isolation into a drop of clean tap water. At the end of 48 hours in preparations containing both oöcysts of this species and of *E. auburnensis*, all of the oöcysts of *E. canadensis* were still in the undivided sporont stage, while those of *E. auburnensis* had developed as far as the elongated sporoblast stage.

*Relationships:* Oöcysts of *E. canadensis* are similar to those of *E. auburnensis* in color, and slightly tapered variants of the former may be confused with smaller specimens of the latter. The oöcysts of *E. canadensis* can be differentiated from those of *E. auburnensis*, however, by having a significantly smaller mean size, greater



ratio of breadth to length and by requiring 24 hours longer for complete sporulation under standard conditions. In addition, the heavily-mammillated wall observed in certain variants of *E. auburnensis* was not seen in oöcysts of *E. canadensis*. In a few oöcysts of this species, however, roughened walls were seen, the roughening consisting of relatively few broad, gently rounded, shield-like plaques instead of the numerous small, sharply rounded elevations characteristic of oöcysts of *E. auburnensis*. While oöcysts with roughened walls probably represent specimens discharged from the tissues of the host before the wall has completely homogenized it is apparent that the tendency for premature discharge of oöcysts is more marked in *E. auburnensis* than in *E. canadensis* or other species of bovine coccidia.

*Occurrence:* Oöcysts of this species were found in fecal samples from several healthy Alabama calves during the course of the early natural infection, but never in great numbers.

In a brief preliminary report, Yakimov (1931) described *E. zurnabadensis* on the basis of oöcysts from the zebu, later (1933) giving a more detailed description accompanied by a figure. The oöcysts were said to measure 25.2 to 43.2 (average 34.1)  $\mu$  long by 18 to 32.4 (average 25)  $\mu$  in transdiameter, were more or less cylindrical in shape, with the two ends equally rounded, and were colorless to yellowish in tint.

In his *E. canadensis*, Bruce (1921) included along with *E. zurnii* and *E. bovis* oöcysts answering the description for Yakimov's *E. zurnabadensis*. He stated that larger oöcysts of *E. canadensis* were always ovoidal or ellipsoidal in shape, including among his figures four of the large, ellipsoidal oöcysts having shapes, dimensions and form indexes within the range specified by Yakimov for *E. zurnabadensis*. Parts of Bruce's *E. canadensis* have been synonymized with both *E. zurnii* and *E. bovis*, but since the remaining part of the description adequately designates oöcysts later described as *E. zurnabadensis*, the name *E. canadensis* should be retained, restricted to apply only to these large, ellipsoidal oöcysts and the name *E. zurnabadensis* reduced to synonymy.

While the average dimensions of oöcysts answering the description for *E. canadensis* from Alabama calves were slightly smaller than those given by Yakimov, the shape index was almost identical. The four specimens figured by Bruce measured 30.7 to 33.2  $\mu$  long by 24.9 to 26.5  $\mu$  in transdiameter, dimensions which place them well within the range specified for the species by Yakimov.

*Eimeria ellipsoidalis* Becker and Frye, 1929  
(Plate I, 3; Plate II, 13)

*Unsporulated oöcysts:* Oöcysts answering the description for this species from Alabama calves measured 12 to 27 (average 16.9)  $\mu$  long by 10 to 18 (average 13)  $\mu$  in transdiameter; 25 to 100 consecutive oöcysts were measured from each of 9 fecal samples from 4 host animals, dimensions being based on a total of 350. Shape predominantly regularly ellipsoidal, but varying from spherical to almost cylindrical, the spherical and subspherical oöcysts occurring in the smaller range; 0.62 to 1.00 (average 0.77) as broad as long. Micropyle imperceptible. Wall thin, homogeneous, transparent, and slightly thinner and paler at one end of the oöcyst to suggest a possible micropyle or weakness in the wall. Under low magnification these oöcysts

appear colorless, but under oil immersion the wall is observed to have a pale lavender to yellowish tint.

**Sporulation:** In 13 trials at room temperature 52 to 100 per cent of the typically ellipsoidal oöcysts sporulated completely within 48 to 72 hours after isolation into a drop of clean tap water.

**Relationships:** Spherical or subspherical oöcysts in the small range of this species may be confused with those of *E. subspherica* or smaller *E. zurnii*, and elongated oöcysts in the larger range of *E. ellipsoidalis* intergrade with oöcysts described for *E. cylindrica*. The standardized sporulation test showed that the oöcysts of *E. ellipsoidalis* require at least 48 hours less for complete sporulation than those of *E. subspherica*. The prevalence of oöcysts of *E. ellipsoidalis* in feces from healthy calves and the frequent association of oöcysts of *E. zurnii* with clinical symptoms of coccidiosis may possibly indicate a significant difference in the pathogenicity of these two species under field conditions. Wilson (1931) has presented fairly convincing experimental evidence that *E. cylindrica* is a valid species, in spite of the tendency for the oöcysts to intergrade morphologically with those of *E. ellipsoidalis*.

**Occurrence:** These colorless, ellipsoidal oöcysts were found in greater frequency and numbers in feces from healthy Alabama calves examined repeatedly during the early natural infection than were those of any other species.

Becker and Frye (1929) described this species from oöcysts found in the feces of a single calf. The oöcysts were 20 to 26 (average 23.4)  $\mu$  long by 13 to 17 (average 15.9)  $\mu$  in transdiameter, were predominantly ellipsoidal in shape, with ovoidal or subspherical forms being rare, and were colorless and more inconspicuous than oöcysts of *E. bovis*. In this study, measurements of several hundred oöcysts from several hosts showed a smaller average size and greater range in size than were recorded by Becker and Frye for oöcysts from a single host. Occasionally oöcysts in a single fecal specimen showed dimensions similar to those given by these authors. For example, 50 consecutive ellipsoidal oöcysts in one fecal sample from a calf measured 17 to 27 (average 22.4)  $\mu$  long by 13 to 17 (average 15.4)  $\mu$  in transdiameter. Three days later, 20 consecutive ellipsoidal oöcysts in a fecal specimen from the same calf measured 16 to 24 (average 19.7)  $\mu$  long by 12 to 16 (average 14.1)  $\mu$  in transdiameter. Apparently the oöcysts in a single fecal specimen from a calf do not represent the entire range for the species. Measurements of oöcysts from calves artificially infected with pure cultures of oöcysts of *E. ellipsoidalis* are needed to define fully the mean size and range in size for oöcysts of this species.

*Eimeria bukidnonensis* Tubangui, 1931  
(Plate I, 6; Plate II, 18)

**Unsporulated oöcysts:** Oöcysts of this species from Alabama calves measured 33 to 41 (average 36.6)  $\mu$  long by 24 to 28 (average 26.7)  $\mu$  in transdiameter; 10 to 25 consecutive oöcysts were measured from each of 4 fecal samples from 3 host animals, dimensions being based on a total of 80. Practically all oöcysts distinctly pyriform, with little variation in shape; 0.63 to 0.80 (average 0.73) as broad as long. Color yellowish-brown to distinctly brown. Wall about 2  $\mu$  thick, discontinuous at narrow end to form a micropyle about 4  $\mu$  in diameter; thickness of wall relatively greater than in any other species of coccidia from bovines.

**Sporulation:** In 4 trials at room temperature 90 to 96 per cent of these oöcysts

sporulated completely within 4 to 7 days after isolation into a drop of clean tap water.

*Relationships:* Oöcysts of this species were easily differentiated from those of all other species of bovine coccidia by their large size, distinctly brown color, thick wall, pyriform shape and long sporulation time.

*Occurrence:* Oöcysts of *E. bukidnonensis* were never found in large numbers or great frequency in Alabama calves, and probably this species has little pathogenic significance.

The specimens observed in Alabama were considerably smaller than those described for the species by Tubangui (1931), his oöcysts measuring 46.8 to 50.4 (average 48.6)  $\mu$  long by 33.3 to 37.8 (average 35.4)  $\mu$  in transdiameter.

*Eimeria cylindrica* Wilson, 1931  
(Plate I, 4; Plate II, 14)

*Unsporulated oöcysts:* Oöcysts answering the description for this species from Alabama calves measured 16 to 27 (average 23)  $\mu$  long by 12 to 15 (average 13.9)  $\mu$  in transdiameter; these dimensions were based on measurements of 66 consecutive oöcysts from a fecal sample in which there were no other morphologically related oöcysts. Shape typically regularly cylindrical, the opposite sides of the oöcyst being nearly parallel throughout the middle third of the long axis; oöcysts vary from ellipsoidal to narrow cylinders twice as long as broad; 0.50 to 0.77 (average 0.60) as broad as long. Micropyle imperceptible. Wall thin, homogeneous, transparent, slightly paler at one end to suggest a possible micropyle. Oöcysts appear colorless under low magnification, but under oil immersion the wall appears slightly tinted.

*Sporulation:* In 2 trials at room temperature 90 and 95 per cent of the typically cylindrical oöcysts appeared to be sporulated within 48 hours after isolation into clean water. The number of tests was not sufficient to be conclusive, but the results indicated that these oöcysts sporulated at a faster rate than those of *E. ellipsoidalis*, which require 48 to 72 hours under identical conditions.

*Relationships:* These cylindrical oöcysts were often found associated with those of *E. ellipsoidalis*, and since morphological intergradation was noted between the two an accurate differential diagnosis was difficult in these mixed discharges. Occasionally, however, the cylindrical oöcysts were found with few or no oöcysts of *E. ellipsoidalis*, and from these it was possible to make what is believed to be a reasonably accurate description of the species. Average oöcysts of the two species are distinct in shape and ratio of breadth to length. The possible diagnostic differences in pathogenicity and in sporulation time between the two groups of oöcysts should be tested further.

*Occurrence:* Oöcysts of *E. cylindrica* were usually found during the initial natural infection in feces from healthy calves examined repeatedly, but with less frequency and in smaller numbers than those of *E. ellipsoidalis*.

On oöcyst morphology alone the distinction between *E. cylindrica* and *E. ellipsoidalis* seems weak, but Wilson (1931) presented additional evidence which indicates that *E. cylindrica* is probably a valid species with pathogenic potentialities. The oöcysts described by Wilson for the species measured 19.4 to 26.8 (average 23.3)  $\mu$  long by 11.9 to 14.9 (average 13.3)  $\mu$  in transdiameter, and were less resistant to freezing temperature and sporulated faster than oöcysts of either *E. zurnii* or *E. bovis*. He recovered typical cylindrical oöcysts in pure discharge from a calf from the eleventh to the twentieth day after experimental dosage, and noted that the feces were streaked with blood on the sixth day after dosage, indicating possible pathogenicity for this species. Additional experi-

mental work is needed to determine accurately the mean size and range in size of oöcysts of *E. cylindrica*, and to clearly differentiate this species from the earlier described *E. ellipsoidalis*. Until more information is available, it seems advisable to retain the name *E. cylindrica* for the oöcysts in question.

*Eimeria auburnensis* Christensen and Porter, 1939  
(Plate I, 9, 10; Plate II, 21, 22)

*Unsporulated oöcysts:* Oöcysts 32 to 45.5 (average 38.4)  $\mu$  long by 20 to 25.5 (average 23.1)  $\mu$  in transdiameter. Shape typically elongated ovoidal, varying between subellipsoidal and markedly tapered; 0.48 to 0.76 (average 0.60) as broad as long. Micropyle a gap in wall at tapered end, covered with a thin, black line which possibly represents an operculum. Wall typically smooth, homogeneous, transparent, and usually noticeably yellowish-brown in tint, but varying in structure from the usual transparent, homogeneous type to a relatively rare, semi-transparent, heavily-mammillated type, with all intergradations of wall roughening between the two extremes; the smooth-walled variants were found in more host animals and usually in greater numbers than the rough-walled variants.

*Sporulation:* In 4 trials at room temperature 91 to 100 per cent of the oöcysts sporulated completely within 48 to 72 hours after isolation into a drop of clean tap water.

*Relationships:* The oöcysts of *E. auburnensis* are differentiated from those of *E. bovis* by their greater size, more intense coloration, relatively narrower and more elongated form, and in their tendency toward the development of the mammillations in the wall. They differ from those of *E. bukidnonensis* in having a thinner wall, paler brownish tint, greater transparency, shorter sporulation time, relatively narrower form, and by the occasional formation of the mammillated wall. They are distinguished from oöcysts of *E. thianethi* in having a smaller average size and thinner wall, and in not showing the transverse striations in the wall as reported for oöcysts of *E. thianethi* by Gwéléssian (1935).

*Occurrence:* Oöcysts of *E. auburnensis* were frequently found in small or moderate numbers in feces from healthy calves. The smooth-walled variants occurred more frequently than the rough-walled forms, which were relatively rare.

This species was described in detail by the writer and Porter (1939) from oöcysts found in feces from several calves from Alabama and a few fecal specimens obtained from Maryland and Montana. In addition to the morphological evidence they presented for the separate identity of *E. auburnensis*, these writers infected a calf with a pure culture of predominantly rough-coated oöcysts of the species and produced a profuse diarrhea from the 9th to 13th days after dosage, followed by heavy discharge from the 24th to the 27th days after dosage of oöcysts showing uniformity in size, shape, color, and sporulation time, and showing a completely intergrading series in wall structure from homogeneous to heavily-mammillated. The potential pathogenicity of the species was shown by the production of scours in this experimental infection.

*Eimeria alabamensis* n. sp.  
(Plate I, 5; Plate II, 15, 16)

*Unsporulated oöcysts:* Oöcysts 13 to 24 (average 18.9)  $\mu$  long by 11 to 16 (average 13.4)  $\mu$  in transdiameter; 7 to 48 consecutive oöcysts measured from each of 10



fecal samples from 4 host animals, dimensions being based on a total of 200. Shape typically pyriform, but showing considerable variation (Fig. 1, C-K); subellipsoidal and subcylindrical oöcysts occur throughout entire range and intergrade perfectly with the majority that are distinctly tapered at one end; asymmetrical forms common in heavier discharges; 0.50 to 0.86 (average 0.71) as broad as long. No micropyle perceptible. Wall thin, delicate, homogeneous, transparent, slightly thinner at narrow end; oöcysts appear colorless and crystalline under low magnification, but under oil immersion the wall is observed to have a grayish-lavender to pale brownish-yellow tint, fading to light yellow at narrow end. Early spherical sporont shows layer of refractile granules in regular arrangement about periphery, giving a beaded appearance (Fig. 1, G).

**Sporulation:** In 5 trials at room temperature freshly discharged oöcysts required 96 to 120 hours for complete sporulation after isolation into clean tap water, 82 to 100 per cent completing the process during this interval. Characteristic of this species was the presence of a parachute-shaped cap at each end of the sporocyst immediately preceding segmentation of the protoplasm into sporozoites (Fig. 1, H).

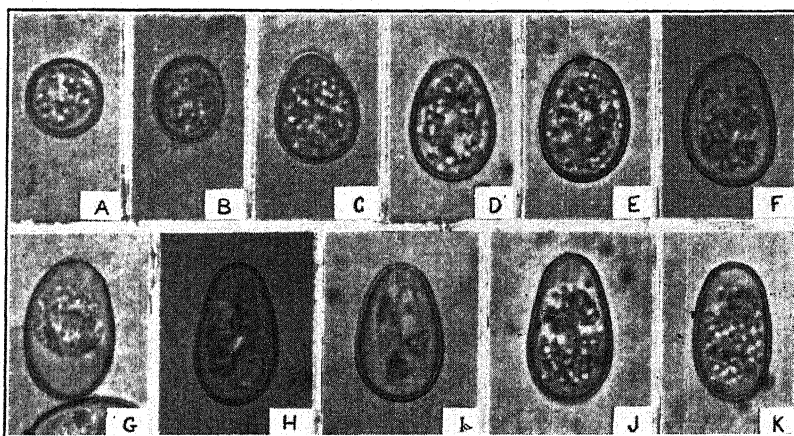


FIG. 1. A, B, oöcysts of *Eimeria subspherica*, showing range of variation in shape. C-K, oöcysts of *E. alabamensis*, showing range of variation in shape. Photographed by Dale A. Porter.

These cap-like structures disappeared upon completion of sporulation. A sporulated oöcyst contained 4 elongated, gently tapered sporocysts, each having 2 indistinct sporozoites. No residual material was observed in either oöcyst or sporocyst.

**Relationships:** In shape the typical oöcysts of *E. alabamensis* resemble those of *E. bovis*, but in mean size they are significantly smaller and sporulation under standardized conditions requires 48 hours longer. Subellipsoidal or subcylindrical oöcysts of this species resemble the oöcysts of *E. ellipsoidalis* or *E. cylindrica* in color, size, and shape, but are easily distinguished from them by requiring at least 48 hours longer for sporulation. After remaining for 24 hours in a hanging drop of the 40 per cent sugar solution used for flotation, the delicate walls of oöcysts of this species were crumpled, while those of oöcysts of *E. bovis*, *E. ellipsoidalis* and *E. cylindrica* remained intact.

**Occurrence:** Oöcysts of *E. alabamensis* were found in small or moderate numbers in feces from several healthy Alabama calves, in one fecal specimen being the only oöcysts present.



*Eimeria subspherica* n. sp.  
(Plate I, 1; Plate II, 11)

*Unsporulated oöcysts:* Oöcysts 9 to 13 (average 11)  $\mu$  long by 8 to 12 (average 10.4)  $\mu$  in transdiameter; 5 to 30 consecutive oöcysts measured from each of 11 fecal samples from 6 host animals, dimensions being based on a total of 115. Shape typically subspherical, varying from spherical to bluntly ellipsoidal (Fig. 1, A, B); 0.75 to 1.00 (average 0.94) as broad as long. No micropyle visible. Wall thin, homogeneous, transparent, of uniform thickness throughout; oöcysts appear pale and colorless under low magnification, but the wall shows a faint yellow tint under oil immersion. These are pallid, fragile oöcysts, and the smallest observed from calves in Alabama.

*Sporulation:* In 5 trials at room temperature freshly discharged oöcysts of this species required 96 to 120 hours for complete sporulation after isolation into clean tap water, 86 to 98 per cent of them completing the process within this interval. In drops containing both oöcysts of this species and of *E. ellipsoidalis*, those of the latter had completely sporulated before those of *E. subspherica* had developed beyond the spherical sporont stage. A sporulated oöcyst of *E. subspherica* contained 4 pale, spindle-shaped sporocysts each having 2 indistinct sporozoites. No residual material was observed in either oöcyst or sporocyst.

*Relationships:* Morphologically these oöcysts may be confused with subspherical oöcysts in the small range of *E. ellipsoidalis* and with smaller oöcysts of *E. zurnii*, but they may be differentiated from these forms by their more fragile appearance, more delicate wall, and by requiring 48 hours longer for complete sporulation under standard conditions. After remaining for 24 hours in a hanging drop of the 40 per cent sugar solution used for flotation, the walls of these oöcysts were crumpled, while those of *E. ellipsoidalis* in the same preparations remained intact.

*Occurrence:* Oöcysts of *E. subspherica* were found in fecal specimens from 6 healthy calves in the vicinity of Auburn, Alabama, never in large numbers. In one fecal specimen they were the only oöcysts present, and in another sample at least 95 per cent belonged to this species. Oöcysts of *E. subspherica* were the only oöcysts in addition to those of *E. zurnii* in the feces of a calf that died of coccidiosis at the age of 5 weeks.

#### IDENTIFICATION OF OÖCYSTS OF BOVINE COCCIDIA

In identification, freshly discharged oöcysts were found to be most typical of a species in color and shape, and for this reason the unsporulated oöcyst is emphasized in this paper. An additional advantage in obtaining fresh fecal specimens was that preparations could be made for determining sporulation times of oöcysts, which provided valuable information in differentiation in certain cases. On the basis of morphology of unsporulated oöcysts and on sporulation times under standardized conditions, oöcysts of bovine coccidia from Alabama calves fell into 9 more or less well-defined groups, 7 of these belonging to established species while 2 are, as already stated, apparently new. In cases where morphological intergradation occurred, differences in sporulation time were helpful in final differentiation. These groups of oöcysts constituted recognizable entities from host to host.

On gross examination, typical oöcysts of species of bovine coccidia observed in Alabama were colorless (*E. subspherica* n. sp., *E. zurnii*, *E. alabamensis* n. sp., *E. ellipsoidalis*, and *E. cylindrica*); almost colorless to pale yellowish- or greenish-brown (*E. bovis*); pale to noticeably yellow-

ish-brown (*E. canadensis*, *E. auburnensis*); distinctly yellowish-brown to brown (*E. bukidnonensis*). In shape the oöcysts were subspherical (*E. subspherica*, *E. zurnii*); ellipsoidal (*E. ellipsoidalis*, *E. canadensis*); cylindrical (*E. cylindrica*); stoutly ovoidal to pyriform (*E. alabamensis*, *E. bovis*, *E. bukidnonensis*); elongated ovoidal (*E. auburnensis*). Colorless oöcysts were smallest in size, tinted ones larger, although under oil immersion even the smallest oöcysts were observed to have some color in the walls, usually varying from pale lavender to light yellow. The wall was homogeneous, thin and transparent in all species except *E. auburnensis* and *E. bukidnonensis*; the heavily-mammillated wall observed in the rough-coated variants of *E. auburnensis* presented a semi-opaque appearance, although in most oöcysts of the species the wall was smooth; the wall in oöcysts of *E. bukidnonensis* was relatively thicker than in any other species and has been described as radially striated, but this was not marked in specimens observed in this study. All oöcysts of bovine coccidia known at present are without polar caps, and micropylar openings were seen only in the larger oöcysts. Only in oöcysts of *E. alabamensis* did the undivided, spherical sporont have a characteristic appearance, the refractile granules arranging themselves in a bead-like layer about the periphery preceding division.

After freshly discharged oöcysts were isolated into small identical amounts of clean water to standardize the environment and were incubated at room temperature, it was found that the time required for complete sporulation varied considerably among the oöcysts of different species. There was some indication that oöcysts of *E. cylindrica* sporulated fastest of all, finishing the process within 24 to 48 hours, although the number of tests with this form was too few to be conclusive. Repeated tests showed that characteristic sporulation times for oöcysts of the other species were 48 to 72 hours (*E. zurnii*, *E. ellipsoidalis*, *E. bovis*, *E. auburnensis*); 72 to 96 hours (*E. canadensis*); 96 to 120 hours (*E. subspherica*, *E. alabamensis*); 96 to 168 hours (*E. bukidnonensis*). These sporulation periods, which were found to be constant for a species once standardized conditions were established, provided valuable accessory information in differentiating oöcysts of *E. subspherica* from subspherical *E. ellipsoidalis*, *E. alabamensis* from *E. bovis*, and *E. canadensis* from *E. auburnensis*.

In the sporulated oöcyst there was little to assist in the differentiation of species. None of the oöcysts showed oöcystic residual material after sporulation; the presence or absence of residual material inside the sporocysts could not be determined with certainty in smaller oöcysts and did not seem to constitute a good differential character. Dimensions of sporocysts were not considered diagnostic and were omitted, since their size is a function of the size of the oöcyst.

Although the 9 groups of oöcysts described here as species are recognizable from morphological and physiological characteristics, there is limited clinical and experimental evidence which indicates that certain of these are probably well-defined species. The frequent association, alone or with few oöcysts of other species, of the subspherical oöcysts described for *E. zurnii* with bloody diarrhea in calves indicates that probably this is a well-defined species with marked pathogenicity. Wilson (1931) recovered oöcysts of *E. cylindrica* in pure discharge from an experimentally infected calf, and the potential pathogenicity was indicated by the appearance of blood-streaked feces on the sixth day after dosage. The writer and Porter (1939) showed that *E. auburnensis* is capable of pathogenic activities by producing severe diarrhea lasting for several days in a young calf by inoculation with a pure culture of infective oöcysts.

Oöcysts of the species of *Eimeria* described in this paper may be differentiated with the aid of the following key:

#### KEY TO OÖCYSTS OF *Eimeria* FOUND IN CALVES

1. Relatively small and colorless; without perceptible micropyle ..... 2  
 Relatively large and tinted; micropyle a gap in wall at one end of oöcyst ..... 6
2. Typical oöcysts subspherical or spherical ..... 3  
 Typical oöcysts pyriform, ellipsoidal or cylindrical ..... 4
3. Relatively small; 9 to 13  $\mu$  long; pallid, thin-walled, delicate; 96 to 120 hours required for complete sporulation ..... *E. subspherica* n. sp.  
 Larger; 15 to 22  $\mu$  long; more conspicuous and crystalline in appearance; 48 to 72 hours required for complete sporulation ..... *E. zurnii* (Rivolta, 1878)
4. Typical oöcysts distinctly pyriform, but varying from subellipsoidal to subcylindrical; 13 to 24  $\mu$  long; pallid, thin-walled, delicate; peculiar parachute-shaped caps at ends of sporocysts preceding sporozoite formation; 96 to 120 hours required for complete sporulation ..... *E. alabamensis* n. sp.  
 Typical oöcysts regularly ellipsoidal or cylindrical; more conspicuous and less fragile than oöcysts of *E. alabamensis* ..... 5
5. Majority of specimens regularly ellipsoidal, varying from spherical to nearly cylindrical; 12 to 27  $\mu$  long; 0.62 to 1.00 (average 0.77) as broad as long; 48 to 72 hours required for complete sporulation.  
*E. ellipsoidalis* Becker and Frye, 1929  
 Majority of specimens regularly cylindrical, varying from ellipsoidal to narrowly cylindrical; 16 to 27  $\mu$  long; 0.50 to 0.77 (average 0.60) as broad as long; 48 hours or less required for complete sporulation. .... *E. cylindrica* Wilson, 1931
6. Typical oöcysts regularly ellipsoidal, with some slightly tapered or nearly cylindrical forms in range of variation; 28 to 37  $\mu$  long; wall homogeneous, delicately yellowish-brown; 72 to 96 hours required for complete sporulation.  
*E. canadensis* Bruce, 1921  
 Typical oöcysts tapered toward micropylar end ..... 7
7. Wall distinctly thickened and yellowish-brown to dark brown in color, these being the most intensely tinted oöcysts noted from bovines; 33 to 41  $\mu$  long; pyriform; 96 to 168 hours required for complete sporulation.  
*E. bukidnonensis* Tubangui, 1931  
 Wall not distinctly thickened or so intensely tinted; 48 to 72 hours required for complete sporulation ..... 8
8. Typical oöcysts stoutly egg-shaped or ovoidal; 23 to 34  $\mu$  long; almost colorless

to pale greenish- or yellowish-brown; wall smooth and homogeneous in all specimens ..... *E. bovis* (Züblin, 1908)  
 Typical oöcysts elongated ovoidal; 32 to 46  $\mu$  long; pale to distinctly yellowish-brown; wall typically homogeneous, but variants occur having numerous, small, rounded mammillations in the wall.

*E. auburnensis* Christensen and Porter, 1939

#### SUMMARY

Nine groups of coccidial oöcysts of the genus *Eimeria* were found in feces from healthy and scouring calves in Alabama, seven fitting the descriptions of established species while two appeared to have been previously undescribed. These oöcysts were differentiated largely on the basis of morphology and sporulation times under standardized conditions. The oöcysts redescribed as *E. zurnii*, *E. bovis*, *E. canadensis*, *E. bukidnonensis* and *E. auburnensis* appeared to represent fairly well-defined groups. Morphological intergradation was noted between oöcysts answering the descriptions for *E. ellipsoidalis* and *E. cylindrica*, but other evidence is presented which indicates the probable specificity of these two forms. Further information, however, is needed to conclusively determine the validity of *E. cylindrica*, the more recently described of the two species. Two new species, *E. alabamensis* and *E. subspherica*, are described from oöcysts which appear to be sufficiently distinct from all other bovine coccidia to warrant specific identity. The names *E. bovis* and *E. canadensis* were considered valid and were retained to define oöcysts later described as *E. smithi* and *E. zurnabadensis*, respectively, which were reduced to synonymy.

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## EXPLANATION OF PLATES

## PLATE I

These figures of oöcysts of bovine coccidia were drawn with the aid of a camera lucida from specimens that had been discharged from the host animals long enough to allow the protoplasm to assume spherical shape. Size relationships are preserved. The projected scale shows dimensions in microns.

1. *Eimeria subspherica*.
2. *E. zurnii*.
3. *E. ellipsoidalis*.
4. *E. cylindrica*.
5. *E. alabamensis*.
6. *E. bukidnonensis*.
7. *E. bovis*.
8. *E. canadensis*.
9. *E. auburnensis*, specimen with homogeneous wall.
10. *E. auburnensis*, specimen with mammillated wall.

## PLATE II

These photographs of oöcysts of bovine coccidia were made from temporary mounts of specimens floated and mounted in 40 per cent sugar solution. Size relationships are preserved. Photographed by Dale A. Porter.

11. *Eimeria subspherica*, unsporulated.
12. *E. zurnii*, unsporulated.
13. *E. ellipsoidalis*, unsporulated.
14. *E. cylindrica*, unsporulated.
15. *E. alabamensis*, unsporulated.
16. *E. alabamensis*, elongated specimen undergoing sporulation, showing characteristic parachute-shaped caps at ends of sporocysts preceding segmentation into sporozoites.
17. *E. bovis*, unsporulated.
18. *E. bukidnonensis*, unsporulated specimen showing characteristic thick wall.
19. *E. canadensis*, unsporulated specimen with regularly ellipsoidal shape.
20. *E. canadensis*, slightly tapered specimen undergoing sporulation.
21. *E. auburnensis*, smooth-walled specimen undergoing sporulation.
22. *E. auburnensis*, unsporulated specimen with heavily-mammillated wall.



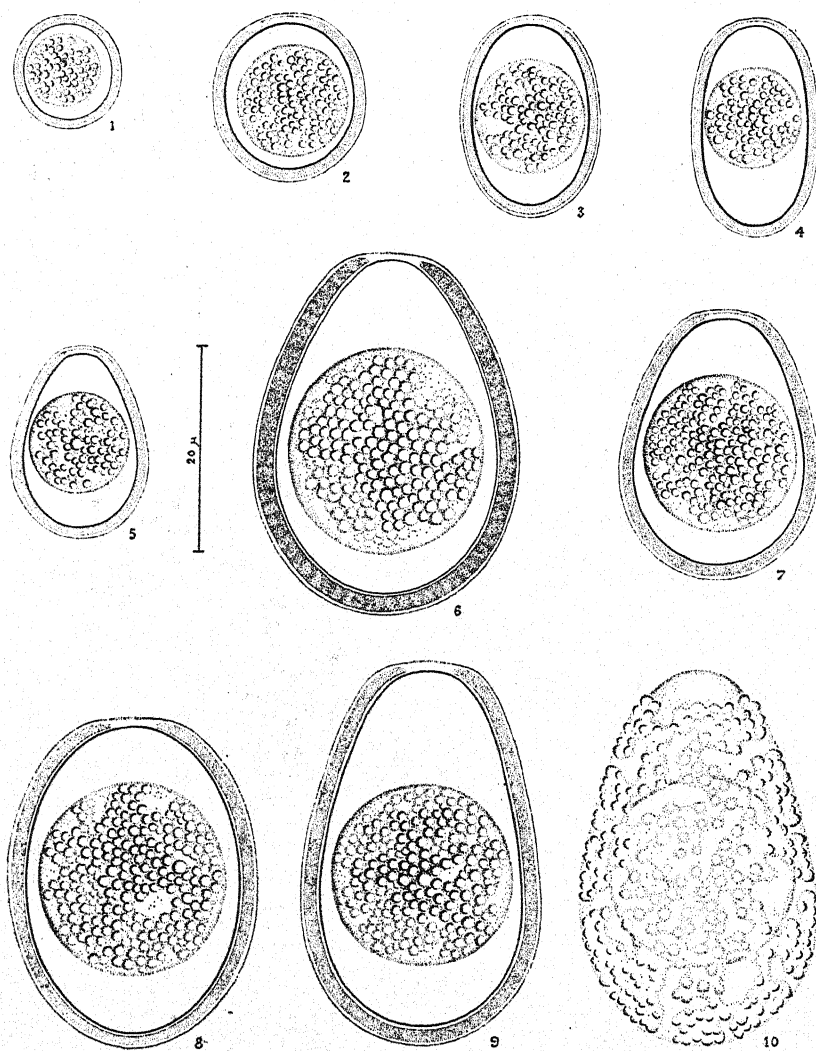


PLATE I

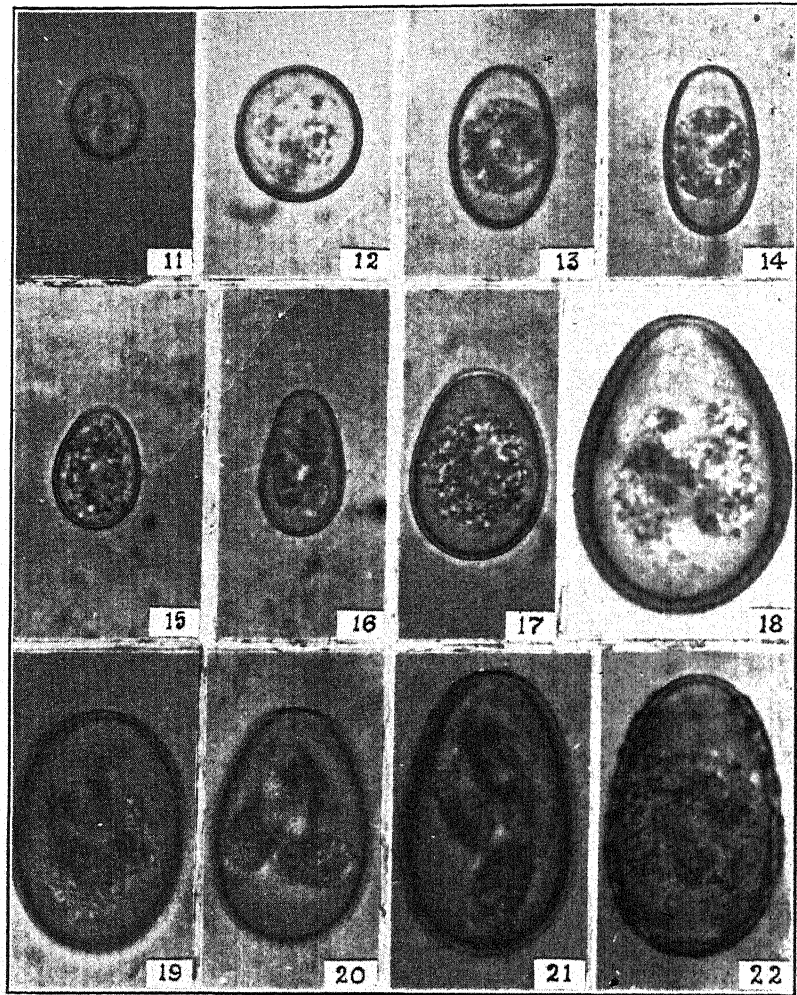


PLATE II

CORALLOBOTHRIUM PARVUM N. SP., A CESTODE  
FROM THE COMMON BULLHEAD, *AMEIURUS*  
*NEBULOSUS* LE SUEUR\*

JOHN E. LARSH, JR.

*Corallobothrium parvum* n. sp. is a tapeworm found in the small intestine of bullheads, first taken in these fish from Black Lake, in the Douglas Lake region of Michigan, the summer of 1939. They were later found in bullheads from southern Illinois.

Fritsch (1886) set aside this genus to accommodate a species of cestode, *C. solidum*, from *Malapterurus electricus*, an electric catfish of Egypt. Fritsch chose this name, *Corallobothrium*, because he thought the scolex resembled an *Oculina*-like coral. Until the work of Riggensch (1896), no other species was referred to this genus. In this work, Riggensch gave the name *Corallobothrium lobosum* to a cestode from *Pimelodus pati*, a siluroid of Paraguay. However, Fuhrmann (1916), in redescribing this tapeworm, disclosed the fact that some of the genital organs were situated in the cortical parenchyma. This fact alone left Fuhrmann with the task of removing this worm from the genus *Corallobothrium*, since all proteocephalids have the entire genital arrangement confined to the medullary parenchyma. Therefore, Fuhrmann created a new genus for this worm, namely, *Rudolphiella*. Fuhrmann and Baer (1925) reduced *Rudolphiella* to synonymy and placed *Rudolphiella lobosum* Fuhrmann in the genus *Ephedrocephalus*. Woodland (1925, part II) proposed to delete such genera as *Corallobothrium*, *Choanoscolex*, *Acanthotaenia*, *Goezeela*, and *Gongesia*, since these groups had been set aside primarily on the basis of the character of the scolex. Woodland gives only secondary importance to this character. Space does not permit one to present evidence for and against the retention of this genus. However, on the basis of the arguments voiced by Essex (1927), Meggitt (1927), and Harwood (1933), and since there is as yet no reliable substitute I propose to follow Braun (1895), La Rue (1914), Fuhrmann (1916), Ward (1918), and other later writers in accepting the genus *Corallobothrium* on the basis of the unusual scolex and the new species described in this paper has been referred to that genus.

*Corallobothrium parvum* n. sp.

(Figs. 1-8)

*Specific diagnosis:* Tetraphyllidean cestode with characters of the family PROTEOCEPHALIDAE and the genus *Corallobothrium*. Length 3.2 mm to 4.2 mm;

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\* Contribution from the University of Michigan Biological Station and the Zoological Laboratory of the University of Illinois. The writer is greatly indebted to Dr. Lyell J. Thomas for his guidance and helpful criticism in this work.

breadth 526 to 683  $\mu$ . Proglottids six to nine, anterior ones wider than long, posterior one longer than wide. Scolex globular, 582 to 631  $\mu$  in diameter, four suckers situated on the anterior surface surrounded by many irregular folds and lappets of tissue (Fig. 6); no rostellum; neither hooks nor spines, genital pores peripheral, irregularly alternating.

Ovary ventral, follicular. Vitelline ducts anastomose anterior to the ovary. Shell gland rosette-shaped, surrounding the oötype. Uterus branching with seven villiform diverticula. Seminal receptacle absent.

Male reproductive system dorsal to that of the female. From eighteen to twenty-five spherical to oblong testes in the medullary parenchyma. Cirrus pouch marginal, irregularly alternating in the anterior fifth of the proglottid, dorsal to the vagina and longitudinal excretory vessels. Vas deferens, at the level of the cirrus pouch, a compact mass of many coils. White colored eggs with three membranes, 18 to 26  $\mu$  in diameter, when mature. Proceroid in *Cyclops*; plerocercoid in the intestine of *Glaridichthys talcatus* and *Ameiurus nebulosus*.

*Type host:* *Ameiurus nebulosus* Le Sueur, the common bullhead.

*Habitat:* The small intestine.

*Type locality:* Douglas Lake, Cheboygan County, Michigan.

*Type specimen:* B. A. I. and U. S. Nat. Museum Helm. Coll. No. 36714.

#### EXTERNAL MORPHOLOGY

These tiny, milky-white cestodes are found attached to the upper end of the host's small intestine in the duodenal region (Fig. 1). Although the strobilization is not perfectly distinct, one is able to detect six or seven proglottids in the chain. Among more than one hundred adult specimens measured before and after fixation, 4.2 mm is the average length recorded. In these, the mature proglottids are about 736  $\mu$  long by 526  $\mu$  wide. The segments in the anterior portion are always wider than long, but proceeding posteriad the length gradually increases at the expense of the width. Thus the posterior proglottids are longer than wide.

The form of the scolex is highly variable owing to different states of contraction (Fig. 2). In living specimens, the size varies from 472 to 578  $\mu$  long by 582 to 631  $\mu$  wide. The four, strongly muscular suckers are directed anteriorly and are more or less concealed by the folds of the scolex. There is no rostellum or rudiment of a fifth sucker revealed in various sets of sagittal and transverse sections. The neck, as seen in sagittal sections, is about 65 to 83  $\mu$  long and 485  $\mu$  wide. It is not easily recognized in much contracted toto mounts.

The cuticula comprises the external body covering. Layers of longitudinal and circular muscles are present. The inner longitudinal muscle sheath is exceptionally well developed in the strobila (Fig. 4). These inner muscular layers make up the boundaries of the medullary parenchyma. The nervous system consists of a ring and two nerve trunks. The former is situated in the median region of the scolex. Arising from the lateral portion of the ring, two trunks pass off parallel to the ascending excretory vessel and follow a course along the inner margin of the longitudinal muscle layer. The excretory tubules are easily seen in sections of immature proglottids. There are four principal longitudinal



trunks which are located in the lateral regions of the medullary parenchyma almost parallel to the longitudinal nerve trunk of each side. In the scolex, these tubules interlock to form a lattice-like network.

#### INTERNAL MORPHOLOGY

A common genital atrium occurs on the lateral margin in the anterior fifth of the proglottid. For the most part, the character and arrangement of the reproductive organs is almost identical with that of other proteocephalids. The entire reproductive system lies within the medullary parenchyma, a constant character of the members of the family PROTEOCEPHALIDAE.

In the male system, the testes are observed to be varied in shape due to pressure exercised by surrounding structures (Fig. 4). The majority are elliptical and measure from 29 to 34  $\mu$  in diameter. From 18 to 25 are present in a single proglottid. They are present in a continuous field between the vitellaria, extending from the anterior margin of the proglottid posterior to the level of the ovary. The vas deferens is prominent; and, at the level of the cirrus pouch, forms a compact mass of many coils that extend almost the entire distance between the vitellaria in the medullary layer. The cirrus pouch is elongate-oval when the cirrus is inverted. At this time, the pouch measures from 136 to 138  $\mu$  long by 45 to 48  $\mu$  in greatest diameter.

In the female system, the vagina opens into the genital sinus ventral to the cirrus. The position of the vagina at this level is almost always posterior to the cirrus pouch. From this level, the vagina passes laterally to an almost median position. From here, it proceeds posteriorly to the region of the genital complex (Fig. 7). The follicular ovary is present in a horizontal anchor arrangement and is situated in the posterior-most region of the proglottid. Along the median line, the vitelline ducts anastomose anterior to the ovary. The vitellaria are compact masses extending in two lateral rows from the anterior to the posterior margin of the proglottid. Surrounding the oötype is the so-called shell or Mehlis gland with its characteristic globular cells. The uterus, in mature and ripe proglottids, is composed of seven or more lateral pouches which give the entire proglottid a wrinkled appearance internally. This structure, in ripe proglottids, almost completely fills the inter-proglottidal space. It extends laterally to the vitellaria and from the anterior margin to near the level of the ovarian isthmus. It opens to the exterior through several uterine pores which fuse into a slit-like cavity in spent proglottids near the ventral median line (Fig. 1).

#### THE LIFE CYCLE

*Observations of the Egg:* After the tapeworms were removed from the small intestine, they were placed in tap water. This change in pres-



sure brought about an expulsion of thousands of eggs. The mature egg is rather small; the size of twenty-three in the first dish was from 18 to 28  $\mu$  in diameter (Fig. 8). The eggs are white and have three membranes. The onchosphere lies beneath the third membrane and may be seen extending and withdrawing its hooks.

*The First Intermediate Host:* A plankton net was used to collect various species of copepods from Douglas Lake. These were placed, a few at a time, in a watch glass that contained a small amount of water. A microscopic examination was made to determine whether they were parasite-free. The non-infected ones were introduced into Petri dishes with the infective tapeworm eggs. In a few minutes, it was observed that many *Cyclops* feasted on these eggs. Twice daily, these copepods were examined for evidence of infection. On the third day, it was observed that the onchosphere had gained access to the body cavity of several *Cyclops*. On the eighth day, the body cavity of *Cyclops prasinus* was found packed with procercoids (twelve in one case). These first larval forms measured, on the average, 342  $\mu$  long by 112  $\mu$  wide. Aside from their constant movement and the presence of calcareous bodies, there was not much detail to be detected (Fig. 5). The packed condition also made it difficult to define the individual boundaries.

*The Second Intermediate Host:* Small tropical fish, *Glaridichthys talcatus*, were used in an experiment to determine if these larval forms would go through another intermediate host. The infected *Cyclops* were placed in a finger bowl with these small fish. In a little while, the fish had ingested eleven *Cyclops*. Three days later, plerocercoid cysts were discovered in the intestinal mucosa and in the body cavity. On an average, these encysted forms were about 158  $\mu$  long by 189  $\mu$  wide. It is not known, as yet, whether this phase of the cycle is a necessary one or merely a storage place for the larval forms until they can be taken by the definitive host. Lack of material did not allow this determination. Until otherwise ascertained, however, it must be assumed that this phase is needed. Hence, it is called the second intermediate host phase in contrast with the unnecessary phase which is termed the auxiliary host phase.

*The Definitive Host:* Seven tropical fish, *Glaridichthys talcatus*, infected with plerocercoids, were placed in a small aquarium with twelve bullheads (about four inches long) which had been purposely starved for some time. The bullheads immediately feasted on these smaller fish. After four days, three bullheads were dissected. The intestine of each fish showed several plerocercoids (Fig. 3). Three of the remaining nine bullheads dissected three days later were found to be infected. The bullheads used in this experiment were taken from a collection of fifty, thirty-three of which were found by dissection to be uninfected. Of the seventeen bullheads remaining from the original collection of fifty, twelve

were selected for this experiment and were presumably uninfected; at least, an infection could be superimposed in this case. The plerocercoids recovered here were opaque, and the body resembled a cone. The calcareous bodies were less numerous than in the proceroid. Aside from this fact and the adult-like condition of the scolex, no other noteworthy facts were observed.

The cycle was thus experimentally completed in *about fifteen days*. From infective eggs to proceroids in the *Cyclops* *about eight days*; from proceroids into plerocercoids in the second intermediate host *about three days*; transfer of plerocercoids into the definitive host *about four days*.

Indications are that other new tapeworms of this interesting genus occur in bullheads that are found in the Douglas Lake region. The present research indicates that ample material will be available. Therefore, further research may provide additional data.

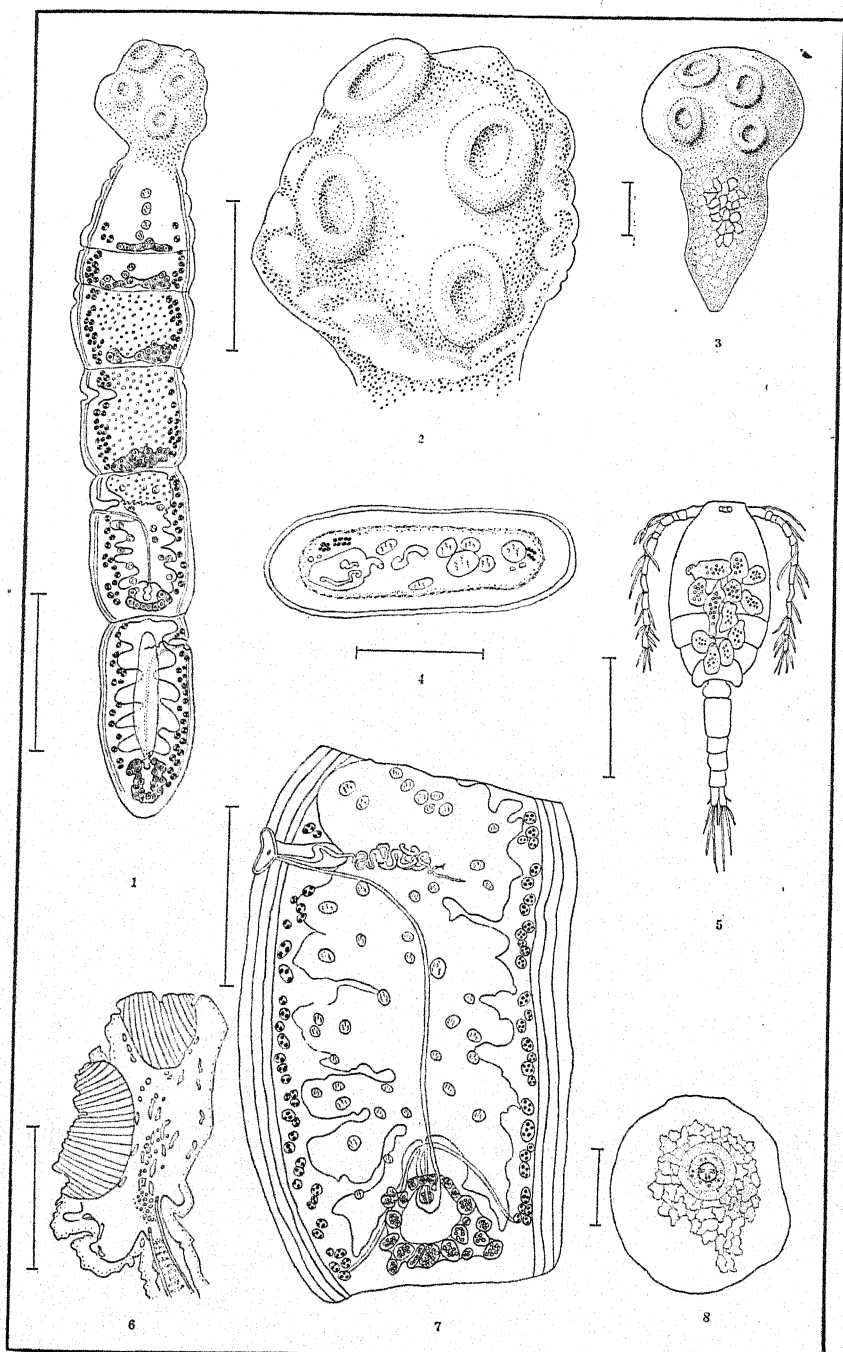
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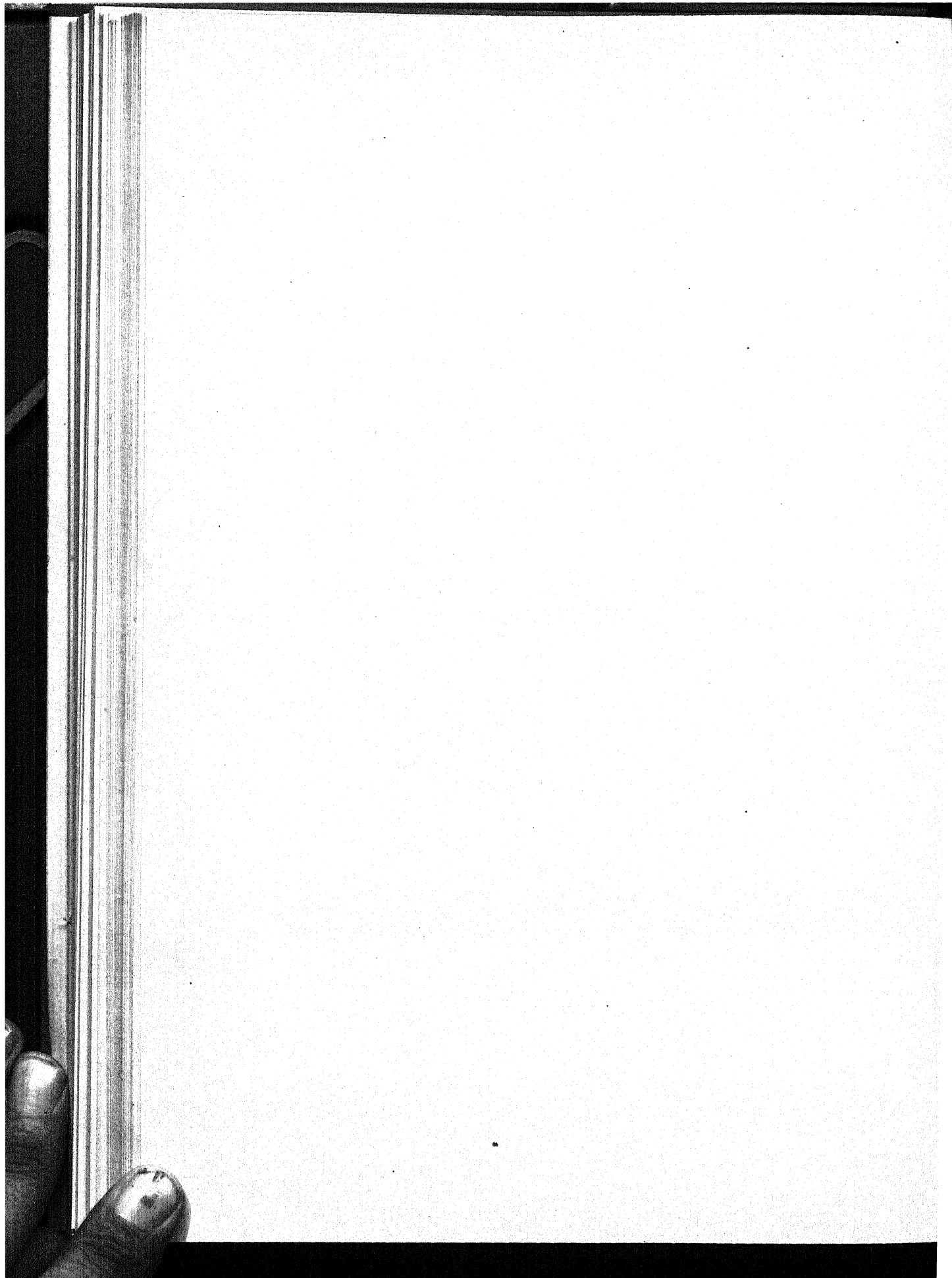
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## EXPLANATION OF PLATE

All figures are camera lucida drawings unless otherwise stated. The projected scale for Figs. 2, 3, 4, 5, 6, and 7 equals 0.03 mm; for Fig. 1 equals 0.20 mm; and for Fig. 8 equals 0.002 mm.

- FIG. 1. Free hand drawing of the adult worm.
- FIG. 2. Dorsal view, toto mount of scolex. A.F.A. fixation.
- FIG. 3. Dorsal view of plerocercoid extracted from the intestine.
- FIG. 4. Cross section through cirrus pouch.
- FIG. 5. Free hand drawing showing dorsal view of *Cyclops* infected with pro-cercoids.
- FIG. 6. Sagittal section through scolex showing portions of the excretory ducts and the fringed margin of the scolex.
- FIG. 7. Free hand drawing of mature proglottid, toto mount, ventral view.
- FIG. 8. Free hand drawing of infective egg soon after laying.







DESCRIPTION AND EXTRAMAMMALIAN LIFE OF  
*CRENOSOMA MEPHITIDIS* N. SP. (NEMA-  
TODA) IN SKUNKS

M. HOBMAIER

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San Francisco, California

A *Crenosoma* was found in *Spilogale gracilis phenax* Merriam and *Mephitis occidentalis* Baird captured in San Francisco and Marin County, California. The parasites inhabit the bronchi below the bifurcation; some may be found on the mucous membrane of the trachea.

*Crenosoma mephitidis* n. sp.

(Figs. 1-2)

*Male*: 8 to 9 mm long and 0.30 to 0.35 mm wide. There are about 20 distinct cuticular folds over a region of about 1 mm of the body length. Parallel longitudinal ridges extend over the entire surface of the cuticle, but gradually decrease in distinctness toward the posterior end of the parasite. Esophagus club-shaped, 350 to 375  $\mu$  long and about 75  $\mu$  wide at its greatest diameter, being only half as wide at its junction with the intestine. A festoon of esophageal cells is inserted into the intestine. Excretory pore opens about 90 to 100  $\mu$  behind head region. Ventral to esophagus and intestine are two large cucumber-shaped cervical glands measuring over 1 mm in length. Intestine fairly straight tube, wider than the esophagus. Bursa well developed (Fig. 1), measuring outspread 300  $\mu$ . Width of body of the parasite above ventral rays 150  $\mu$ . Bursa notched at the ends of the ventral, posterolateral and dorsal rays. Rays springing from stout bases and well developed. Ventral rays only partially divided. Ventrolateral stouter and longer than the ventroventral. Externolateral closely situated to medio- and posterolateral, as is the externodorsal. Externolateral and externodorsal of similar appearance; both end near margin of bursa. Mediolateral and posterolateral divided about half their lengths. Dorsal simple. It shows at least 2 minute papillae distally. Spicules equal in size, not over 380  $\mu$  long. Viewed dorsoventrally they appear straight; laterally seen they are slightly bent proximally. Tapering distally they end in a small knob. Both spicules closely situated together, covered by a delicate cuticular sheath, strongly connected by a chitinized thorn with a bifurcated 90  $\mu$  long accessory piece.

*Female*: about 18 mm long and 0.6 to 0.7 mm wide. Cuticular folds extend over an area of 2.60 to 2.75 mm. Size, structure and accessories of esophagus similar in both sexes. Vulva opens about 1 mm above equator of body length. Opening of vulva simple retraction of cuticle without appendices. Vagina slightly chitinized, inserted at the middle of the kidney-shaped ejaculatory duct. Ovejector parallel to longitudinal axis of body; other parts of genital organs double as usual. Tail (Fig. 2) 140 to 150  $\mu$  long, pointed, 2 minute lateral papillae 20  $\mu$  distant from tip of tail. The thin-shelled, colorless eggs 72  $\mu$  in length and 36  $\mu$  in width. They contain fully developed first stage larvae.

*Host*: *Spilogale gracilis phenax* Merriam, and *Mephitis occidentalis* Baird.

*Location*: Bronchi.

*Locality*: San Francisco and Marin County, California.

*Type specimens*: Hooper Foundation, San Francisco.

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Comparing this *Crenosoma* with other known species in the tabulation given by Buckley (1, p. 236) there are differences noticeable. Its similarity, however, to *Crenosoma vulpis* is manifest. Differences between both are seen in the slightly larger size of the *Crenosoma* of the skunk, especially in the female, in the presence of cervical glands, and in the position of the anus closer to the tip of the tail. The rays extend nearer to the border of the velum, and this parasite is not strictly viviparous. Further investigations possibly may show that presence of cervical glands and deposition of embryonated eggs are generic in *Crenosoma*.

#### DEVELOPMENT OF INFECTIVE LARVAE

Larvae still enclosed in the eggshell become livelier as the egg moves to the stomach. The shell becomes softened and is extended and retracted by the movements of the larvae. The shell is finally dissolved without residue. Larvae, as a rule, are freed at their arrival in the small intestine. They are easily demonstrable in stools and they may be isolated by the filter method (2).

Newly hatched larvae (Fig. 3) measure alive 270 to 305  $\mu$  in length by a maximum width of 14  $\mu$ . The esophagus is 110 to 120  $\mu$  long and 6.5  $\mu$  wide near the posterior end. The genital primordium is situated at the middle of the intestine. The length of the pointed tail is about 35  $\mu$ .

Larvae may be kept alive in water for several weeks, if the temperature is below 15° C and putrefaction is strictly avoided. Dried on glass slides, a few larvae revived in water after 5 to 6 weeks.

As in the case of *Crenosoma vulpis* (3) a great variety of slugs and snails are found to serve as intermediate hosts. Infective stages could be raised in different species of *Limax*, *Agriolimax*, *Milax*, *Helix* and in *Epiphragmophora* at our disposal. The larvae are seen enclosed in nodules embedded in the foot of the mollusks, as well as in internal organs.

The infective larva is 525 to 560  $\mu$  long and 25 to 29  $\mu$  wide. In a larva (Fig. 4) 525  $\mu$  long the esophagus measured 210  $\mu$ . There seem to be two lips forming the anterior end. A small buccal capsule leads into the chitinized muscular part of the esophagus, which is 55  $\mu$  long. The glandular part, 150  $\mu$  long, shows no special bulb formation, becoming gradually wider nearing the junction with the intestine. The excretory pore opens 135  $\mu$  behind the anterior end. The width of the parasite at the junction is 25  $\mu$ , near the middle of the body it is 29  $\mu$ , from which it decreases gradually toward the anus. The tail measures 43  $\mu$  in length. The larva tapers less posteriorly than it does anteriorly and ends in a minute spine. The intestine is connected with the anus by a cuticular rectum 18  $\mu$  in length. The genital primordium is visible in the middle of the length of the intestine.

Formation of infective stages requires from 4 to 5 weeks. Two molts are observed during this development and as usual none of the sheaths is shed. There is evidence that larvae with two sheaths are not necessarily infective, as demonstrated by the following observation: Tissues of infected intermediate hosts were exposed to artificial digestion. Investigation of the fluid about half an hour later disclosed the presence of freely moving unsheathed larvae, apparently representing fully developed infective stages. There were, however, also dead larvae encountered enclosed in two sheaths. The latter seemingly were not able to withstand digestion, having not yet attained their full development. This simple method of determination of the viability of third stage larvae may occasionally be useful in place of tedious feeding experiments.

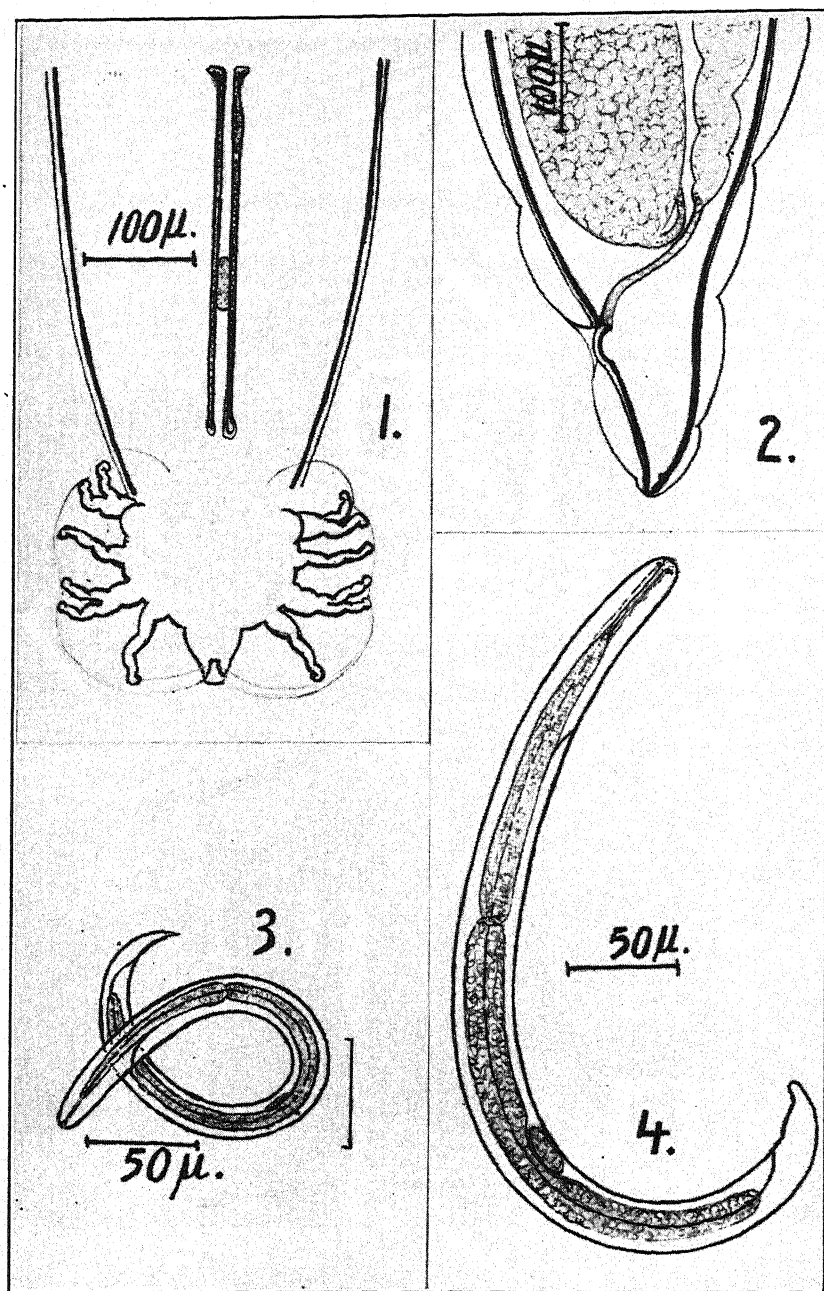
The Pacific garter snake, *Thamnophis sirtalis infernalis* Blainville, was found to serve as a natural auxiliary host. Parasitic tubercles, even visible to the unaided eye, may be seen covering the outside of the stomach and of the intestine. Microscopic investigation reveals their presence in even larger numbers in and under the mucous membrane of both organs. Few nodules only are encountered in the body cavity. No alteration of the parasites was seen in a snake kept in captivity for 12 months. Practically no calcified nodules were found in all the 38 snakes naturally infected. It is likely that the difference in localization of the parasitic cysts is caused chiefly by the increasing tissue resistance built up with the progressing age of the snakes. Other poikilothermous animals, namely frogs, toads and lizards, could be artificially infected. The larvae were found chiefly in and under the mucous membrane of the stomach. The intestine itself contained the parasites in lesser numbers.

Skunks kept in the animal house lost infections after several months, but infections of *Crenosoma mephitidis* and of *Skrjabinogylus chitwoodorum* still persist in skunks living in pens outdoors since 1935. This is explained by the possibility of reinfections in the pens.

Control of *Crenosoma* has to take in account the possibility of spread of the parasite by poikilothermous animals. This may be of concern in combating *Crenosoma vulpis* on fox farms.

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*Crenosoma mephitidis* n. sp.

1. Posterior end of male.
2. Posterior end of female.
3. First stage larva.
4. Third stage larva.

EXTRAMAMMALIAN PHASE OF *PHYSALOPTERA*  
*MAXILLARIS* MOLIN, 1860 (NEMATODA)

M. HOBMAIER

George Williams Hooper Foundation, University of California,  
San Francisco, California

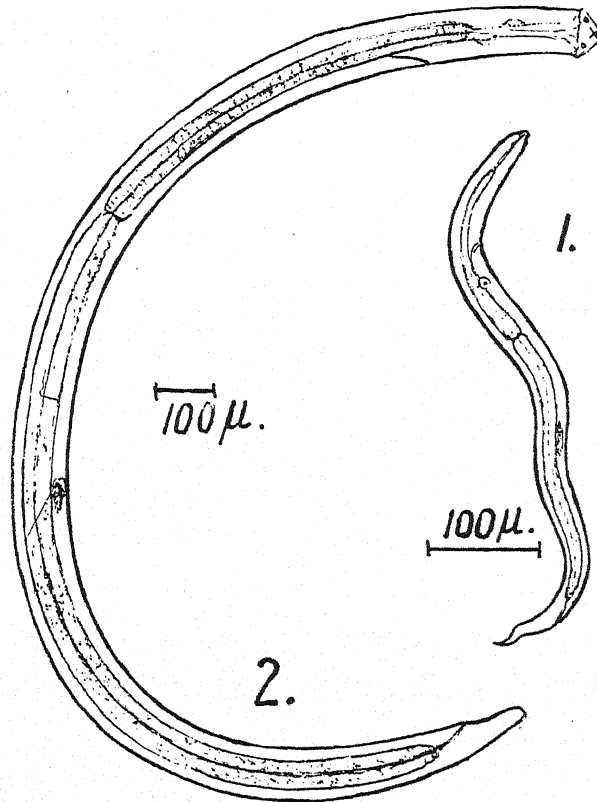
Freshly deposited eggs contain, as already known, a fully developed larva which does not hatch outside the intermediate host. No opercula are visible on the eggs. The vitelline membrane, however, becomes dissolved at the poles of the egg under the influence of digestive juices and calottes of the thick outer eggshell consequently become detached. The middle part of the eggshell, shaped like a barrel with slightly fringed edges, remains unaltered. This procedure takes place in fertile eggs as well as in sterile ones. The larva takes no part in liberating itself.

Living first stage larva are from 275 to 310  $\mu$  long and about 15  $\mu$  wide. Slightly attenuated anteriorly they end in a small cap-like head structure. Posteriorly they taper slowly but continuously to end in a pointed simple tail. The esophagus is 110 to 130  $\mu$  long. The excretory pore opens 75 to 90  $\mu$  behind the anterior end of the body. Beneath the pore a transparent vesicula may be seen. The tail is about 35 to 50  $\mu$  long (Fig. 1).

Further development of the larva is similar to that of *Physaloptera turgida* (1). The cockroach, *Blatella germanica*, was used as intermediate host. To keep and to raise cockroaches cages or containers may be used and water may be provided as described in Galtsoff et al (2). A piece of crumbled paper placed into the containers serves as hiding place. Dry sugar is given as only food. This arrangement prevents mold formation, requires a minimum of care, and provides satisfactory conditions for parasitic experiments. Moldy water or food may develop an aspergillosis in the cockroaches, which causes the inner organs, together with the parasites present, to undergo dry necrosis.

Infective larval stages are attained in from 4 to 6 weeks. Their final sizes vary from 1.8 to almost 3 mm in length by a maximum width of 120  $\mu$ . These differences are due only in part to age and sex. The following observation may explain part of the phenomenon: Esophagus and intestine appear usually as a fairly straight tube. Occasionally, however, the cuticle expands insufficiently. Consequently, the esophagus is bent S-shape and the intestine folded. Such larvae, of course, remain in measurements below their actual sizes. One or more larvae may be found enclosed in colorless cysts according to the severity of the infec-





*Physaloptera maxillaris*

1. Freed first stage larva.
2. Third stage larva.

tion. Some of the cysts may show a golden-brownish color similar to that of the cuticle of the cockroach, with or without destruction of the enclosed larva. In lungworm larvae in snails, where we observed similar colorations occasionally, pigmentation may be observed beginning in the larval sheath prior to or in the process of molting.

Larvae fed to larvae of *Tenebrio molitor* were found six weeks later in the abdominal cavity. They had died without further development and were intensely brownish colored. Such pigmentations as observed in *Blatella* and *Tenebrio* were absent in the natural intermediary host *Stenopelmatus longispina*, Brunner. It is concluded that pigmented incrustations result from host tissue reactions following invasion of less suitable intermediate hosts by parasites.

Third stage larvae of *Physaloptera* (Fig. 2) are readily distinguishable from similar stages of other nematodes in cockroaches by the characteristic formation of their head parts. As in adult *Physaloptera* these

are already triangular in outline. The delicate structure of two lips with teeth, papillae, and even amphids are already discernible. In a living larva, 2.13 mm long with a maximum width near the esophageal junction of  $110\ \mu$  the proesophagus was  $170\ \mu$  long, the postesophagus  $670\ \mu$ , the intestine 1.13 mm, the tail  $160\ \mu$ . The distance from the anterior end to the nerve-ring measured  $150\ \mu$ , to the excretory pore  $180\ \mu$ . The horseshoe-shaped genital primordium was situated just anterior to the middle of the intestine. The tail ended bluntly.

No parasites were found on examination 6 weeks after having fed infected cockroaches to cats, dogs and guinea pigs. Adult stages of *Protospirura muris*, on the other hand, were obtained under similar conditions in guinea pigs, and subsequent infections of intermediate and of final hosts could be carried out.

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EXTRAMAMMALIAN PHASE OF *SKRJABINGYLUS*.  
*CHITWOODORUM* (NEMATODA)

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This nematode has been reported and described recently by Hill (1) as a parasite of *Mephitis mesomelas mesomelas*. Hill gives the size of the female parasite as 29 mm by 660  $\mu$ . We found fully grown specimens 42 mm long and 850  $\mu$  wide in infections of *Spilogale gracilis phenax* Merriam and *Mephitis occidentalis* Baird, observed in the bay region of San Francisco, California. Collection of the parasites is facilitated by resecting the roof of the frontal sinus—their natural habitat—and then submerging the skull in water. Most of the nematodes will voluntarily emigrate into the water under this condition. The nematodes withstand bursting by osmosis for several days. The larvae in the uterus remained alive and freely moving during this time. Larvae dried on blotting-paper did not revive after two days.

First stage larvae are discharged with the feces. In post mortems they may be recovered from the inner surfaces of the entire respiratory and digestive tract. No expulsion of larvae by nose was seen. Demonstration and collection of these and similar larvae from stools may be facilitated by the use of the following method: Feces are comminuted in water and strained into a glass jar. Its top is now covered with three to four layers of gauze. A funnel closed on its end with an adjustable glass tube is filled with water to receive the inverted glass jar. After standing undisturbed for a few hours the larvae present will collect in the adjustable glass tube in a fairly clean condition (2).

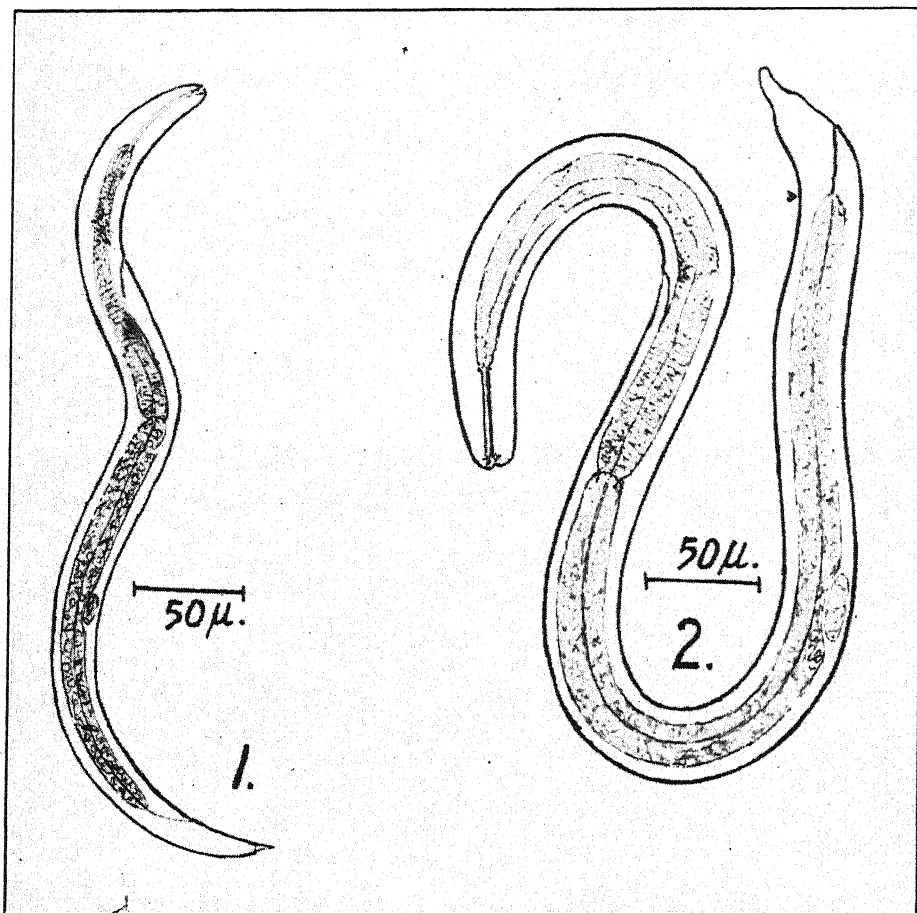
Larvae measured alive (Fig. 1) were 410 to 450  $\mu$  long with a width of 13  $\mu$  to 15  $\mu$  at the esophageal junction. The larva tapers continuously and not abruptly behind the tail to end in a short spine. Neither stained nor unstained specimens revealed distinctive features of the head parts. Granules partly cover internal structures of the larva. The esophagus is 160 to 180  $\mu$  long, widening distally to a small bulb about 10  $\mu$  in width. The excretory pore opens approximately 100  $\mu$  distant from the head region, just below the nerve ring. The genital primordium is situated near the middle of the intestine. The tail measures about 43  $\mu$  in length. The larvae are livelier and taller than those of *Crenosoma*.

Third stage larvae developed in all the slugs and snails available at the time of the experiments, namely: *Limax maximus*, *L. cinereus*, *L.*

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*flavus*, *L. niger*, *Agriolimax agrestis* and *Milax* sp., while *Epiphragmophora* and *Helix pomatia* seemingly were less favorable hosts. The larvae are enclosed in tubercles situated mostly in inner organs of the mollusks. The foot region seems to be a less favored site of invasion. Infection seemingly takes place by active invasion of the foot as well as



*Skrjabinigylus chitwoodorum*

1. First stage larva.
2. Third stage larva.

following intake of food. The latter conclusion is based on the observation that the intestinal wall itself occasionally becomes heavily infected. The larvae often liberate themselves during investigations, abandoning nodules and sheaths. Once freed they show great agility. Preliminary experiments indicate that the route of immigration in final hosts simply



follows the nasal passages. Almost instant liberation and great activity of the larvae are necessary to achieve this goal before the infected food has been swallowed. The thorough mastication of the food noted in skunks facilitates freeing of the larvae.

The larvae are considerably longer and wider than those of *Crenosoma* (Fig. 2). They measure (alive) 830 to 850  $\mu$  in length, with a maximum width of 36  $\mu$  behind the esophageal junction. The esophagus, about 290  $\mu$  long, is club-shaped, becoming only moderately wider distally, without presence of a distinct bulb. The excretory pore opens approximately 110  $\mu$  behind the head region. The parasite is more attenuated anteriorly than it is posteriorly. The tail is only 40  $\mu$  long. The genital primordium is situated on the intestine near its middle measuring about 30  $\mu$  in length. The straight intestinal tube is connected with the anus as usual by a short cuticular rectum.

No adult stages could be raised in experiments on four guinea pigs, two ferrets, three dogs and three cats. In mice and rats some of the larvae could be recovered from the wall of the stomach and its mesenteries. The larvae disintegrated, however, during the second or third week.

Natural infection by third stage larvae was observed in the Pacific garter snake, *Thamnophis sirtalis infernalis* (Blainville). Localization of the larvae was similar to that of *Crenosoma*. Frogs could be artificially infected. Poikilothermous animals therefore serve as intermediate hosts, and rats and mice may serve occasionally as accidental transitory carriers of infective stages. For identification of the snake I am indebted to Dr. C. M. Wheeler. From autopsies it was learned that the main food of the Pacific garter snake consists of slugs. *Limax flavus*, the largest and most common slug of the San Francisco region often formed its exclusive stomach content.

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THE SPECIFIC STATUS OF *MONILIFORMIS* (ACANTHO-  
CEPHALA) OF TEXAS RATS, AND A REVIEW OF  
THE SPECIES OF THIS GENUS IN THE  
WESTERN HEMISPHERE

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The writer (1921) reported the common occurrence of a species of *Moniliformis* in various species of *Rattus*, particularly *R. alexandrinus*, in Houston, Texas and expressed uncertainty as to the relationships of these worms to various previously described members of the genus, both in this country and in Europe. Van Cleave (1925), after examination of some of the Texas forms and comparison of them with specimens from various other parts of the world, came to the conclusion that they belonged to the type species, *M. moniliformis* (Bremser, 1811), and that there was only one other valid species in the genus, *M. clarki* (Ward, 1917) from American squirrels and a mole. Meyer (1933) reviewed the genus again and recognized no less than nine species. He considered the Texas form distinct and named it *M. dubius*, and also considered a form from Brazilian rats, reported by Travassos (1917), as distinct and named it *M. travassosi*. Another form, obtained from the droppings of a Brazilian vampire he also recognized as a separate species, *M. convolutus*.

Since the Texas form has been extensively studied by the writer and his students since 1921, and since there were certain errors in the original account of it which have influenced Meyer in his taxonomic conclusions, and have been widely quoted by other authors, it is desirable to amend the account and bring it up to date, and to discuss the specific status of this and other American forms.

The body length of mature female worms was given in 1921 as 140 to 270 mm and of mature males as 30 to 45 mm. Van Cleave (1925) made a point of the body size in distinguishing *M. moniliformis* from the smaller *M. clarki*. A study of the host-parasite relations of the Texas worms by Burlingame and Chandler (1940) showed that body size is enormously influenced by the age of the worms, the number of worms harbored, the presence of worms from a prior infection, the position of the worms in the intestine of the host, and the host itself, even within a single species (albino laboratory rats). The size of sexually mature worms may vary from 60 mm for females and 30 mm for males in 7-week-old worms in secondary infections, to 320 mm for female and 145 mm for males in 6-month-old light primary infections. Unless the history of an infection is

known, therefore, body size cannot be given much weight in separating species.

It is otherwise with the size and structure of the proboscis. This organ is fully developed in the infective larva before infection of the final host, and so is not influenced by the factors which determine body size. Some variation may occur due to contraction, and some invagination of the tip of the proboscis may be overlooked, but in specimens with the proboscis fully extended the variations due to contraction are slight. In measurements of a large number of the Texas worms, including both infective larvae from the body cavity of roaches and adult worms from the intestines of wild rats and of experimentally infected albino rats, the length of the proboscis varies from 550 to 640  $\mu$ ; it is occasionally almost cylindrical in larvae, but in adults is always somewhat spindle-shaped, with the maximum diameter about one-third to one-half the distance from apex to base. Measurements of proboscis diameter in numerous specimens fall within the following limits: near apex, behind first row of hooks, 160 to 175  $\mu$ ; maximum diameter, 190 to 240  $\mu$ ; base, 160 to 185  $\mu$ .

The number of longitudinal and transverse rows of hooks is sometimes difficult to determine, since the arrangement is not quite regular. There are, however, in all specimens I have examined, larva or adult, 12, 13, or 14 longitudinal rows, with usually 10 or 11 hooks in each row, rarely 9 or 12, making a total of about 125 to 145 hooks. Both my 1921 description and Van Cleave's 1925 description are erroneous with respect to this particular character, although Van Cleave's figure is correct.

The size of the eggs, if they are fully mature, varies between 112 and 125  $\mu$  in length and between 55 and 62  $\mu$  in diameter. The thick inner shell measures 85 to 90  $\mu$  by 36 to 38  $\mu$ , and the embryo itself, enclosed in the egg, 78 to 85 by 28 to 30  $\mu$ .

Some preliminary attempts (Chandler, 1921) to infect cockroaches were reported negative, but since then we have had no difficulty in obtaining development in *Periplaneta americana*, and find a high percentage of naturally infected roaches in places where infected rats are known to occur. This coincides with Travassos' (1917) observations in Brazil, and it is probable that the larvae described by Southwell (1922) from roaches from Gold Coast, Africa, belong to this species.

If the characters of the proboscis and eggs of this form are compared with those given for the type species as determined by Meyer (1933) for some of Bremser's original material, the following differences are noted: (1) the proboscis is much larger, and is spindle-shaped rather than club-shaped; (2) there are 10 or 11 instead of 7 or 8 hooks in each of the 12 to 14 longitudinal rows; (3) the hooks are larger and do not gradually diminish posteriorly, although those near the base of the proboscis are smaller than the others; (4) the dimensions of the ripe eggs are from  $1\frac{1}{2}$  to 2 times greater.

There can be little question that these differences are of specific value. Since the Texas form is also distinct from *M. clarki*, which more closely resembles *M. moniliformis* than it does our worm, and since it does not appear to be specifically identical with any of the other forms described prior to 1933, Meyer's name *dubius* appears to be valid. There are, however, no specific points of difference, as Meyer thought there were, between this worm and the one described from rats in Brazil by Travassos (1917), so *M. travassosi* Meyer, 1933, is considered a synonym of *dubius*. *M. dubius* has also been reported by Yamaguti and Miyata (1938) from rats on ships in Japan. Tubangui's (1931) description of *Moniliformis* from *Rattus norvegicus* in the Philippines leaves little room for doubt that he also was dealing with *M. dubius*. The *Moniliformis* recorded from *Rattus* spp. in Sydney, Australia (Johnston, 1918), Canton, China (Chen, 1933), Washington, D. C. (Price and Chitwood, 1930), and Baltimore, Md. (Luttermoser, 1936), all recorded as *M. moniliformis*, are probably also *M. dubius*. This species appears to have a world-wide distribution, having been spread by its cosmopolitan, sea-going intermediate and definitive hosts, whereas the other species may have much more restricted ranges.

It is probable that *clarki* is a valid species, although in many respects it closely resembles *moniliformis*; it has a more slender proboscis and a larger number of hooks, according to Van Cleave's figure. Meyer's *convolutus* is in most respects very close to *dubius* but differs in that the proboscis hooks have forked roots (see Meyer, 1931, fig. 250).

Sandground (1926) reported the presence of juvenile forms of *Moniliformis* in toads and lizards in Central America. Sandground assumed that these were incidental parasites but that they had taken up a truly parasitic existence, since many were firmly attached to the walls of the intestine. He remarked that the worms had attained a stage considerably in advance of those previously described from cysts of insects, but this is only because fully developed larvae had not been described up to that time. The photomicrograph of a larva of *M. dubius* from a cockroach in Burlingame and Chandler (1941) and the drawing of it in Chandler (1921) represent a larva in the same stage of development as the male larva figured by Sandground. When larvae of *M. dubius* were fed to toads in our laboratory, however, no temporary attachment of the parasites occurred, so it is possible that the toads and lizards may have something more than an accidental relationship to some Central American species of the genus.

Although Sandground referred these young worms to the species *moniliformis*, it is evident that they cannot belong to that species, since the proboscis hooks are of different shape, size and arrangement, and the proboscis more slender. These characters also distinguish it from both



*clarki* and *dubius*. It will probably prove to belong to a new species of which the adults have not been found.

In summary the genus *Moniliformis* is represented in the Western Hemisphere by the following species:

*M. clarki*, in *Sciurus*, *Citellus* and *Scalopus*, in Midwestern States of North America.

*M. dubius* (syn. *M. travassosi*) in *Rattus* spp., cosmopolitan.

*M. convolutus*, in *Vampyrus*, in Brazil.

*M. sp.*, juvenile, in toads and lizards, in Central America.

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## AVIAN TOXOPLASMOSIS WITH INVASION OF THE ERYTHROCYTES<sup>1</sup>

REGINALD D. MANWELL

*Toxoplasma* is a genus of parasites about which very little is known. Wenyon (1926) groups them with several other genera as "parasites of doubtful nature" and Sabin and Olitsky (1937) speak of the "as yet ill-defined group of Protozoa called *Toxoplasma*." A few authors questioned, at least until recently, the existence of the group at all. At present however more attention is being given *Toxoplasma*, partly because it has been shown that parasites belonging to this genus may cause encephalomyelitis in man (Wolf, Cohen and Paige, 1939) and partly because the unpigmented or exoerythrocytic stages of malaria may bear a considerable resemblance to these forms, so that there has been some question as to whether the two stages of malaria found in the vertebrate host did not represent a mixed infection of malaria and *Toxoplasma*.

The usual definition of *Toxoplasma* states that it is a genus of intracellular parasites, occurring in various types of leucocytes, in the body fluids, and even in other types of tissue, notably in the endothelium. They may be found in large numbers in various organs, particularly in the liver, spleen, bone marrow, lungs, kidneys and brain. The parasites themselves are small, and usually crescent-shaped. The accepted mode of reproduction is binary fission, although schizogony has also been claimed to occur.<sup>2</sup>

In a recent paper Sabin (1939) suggests that "the capacity to multiply and to produce disease in a variety of hosts, including mammals and birds, must, in accord with the conclusions of Aragao (1933), be regarded as the chief taxonomic characteristics of the group. Morphology as the only guide can be misleading and confusing. . . ." But it seems to the author that to make the absence of a strong host-specificity the chief characteristic of a genus only adds to the confusion, for this is certainly a relative matter. It is surely conceivable that different species may exist in the genus, and that these are likely to vary in the degree of host-specificity they exhibit. For these reasons it seems better to base the identification of the avian parasites which have been classed as *Toxoplasma* on morphology than to regard them as something else because transmission to other hosts has not

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<sup>2</sup> Raffaele (1932, Riv. Malariol. 11) describes schizogony in avian toxoplasmosis, and figures a number of stages which appear to form parts of a rather complex life-cycle. Although it seems certain that he was dealing with parasites which others have called *Toxoplasma* it is clear that they were in fact different, for binary fission was apparently not observed.

yet been accomplished. The point may readily be conceded that as more knowledge of this group accumulates it may become desirable to change their taxonomic classification, even to the extent of regarding them as something other than protozoa.

The present note is concerned chiefly with an infection with *Toxoplasma* which was recently seen in an English sparrow, and which was unique in that invasion of the red cells occurred on a considerable scale. The diagnosis was made on the basis of the morphology of the parasites and their distribution in the organs. Neither any thing seen in the case itself, nor the results of subinoculation into canaries gave any reason to think the infection might have been mixed. The fact that the bird was only a fledgeling, not more than three or four weeks old, makes it still less likely that more than one species of parasite was concerned. It was also clearly an acute infection, and the death of the bird two or three hours after being brought into the laboratory in a comatose condition suggests that this disease may be one of importance in nature, particularly in young birds. We have also observed many infections of toxoplasmosis among adult English sparrows, among which it seems to spread rapidly after they are brought into the laboratory and kept in relatively close quarters. Not infrequently the disease proves fatal, but there are often chronic cases.

Autopsy of the bird showed a very dark and much enlarged liver, and a spleen of similar appearance. The latter looked much as it often does in an acute case of malaria. The volume was approximately 450 cmm, perhaps 20 times the normal size. Other organs were negative. Smears of different organs did not show large numbers of parasites except in the liver and spleen, where they were extremely numerous. No pigment was observed, a fact which is further evidence that there was no malaria present.

The most interesting observation in connection with the present infection was the occurrence of the parasites within the red cells. In this situation they resembled malaria plasmodia markedly except that within the host cell the crescent shape was obvious, and pigment was lacking. In a few cases there were small granules which could have been mistaken for pigment (in Fig. 1, the arrow indicates such a granule). It was at first thought that the infection was either one of malaria, or a mixed infection of malaria and *Toxoplasma*. Examination of the organs showed that neither of these possibilities could be true, and despite hours of searching no parasites could be found in the blood (except in the leucocytes, or free) which were undergoing reproduction, or anything resembling schizogony. The staining reaction also differed from that seen in malaria in that it was more intense when Giemsa was used.

The microphotographs in the accompanying plate show parasites in different situations or stages. Figs. 1 and 2 depict forms in erythrocytes.

The question might arise as to whether these parasites were actually in the cells, as they appear to be, or whether perhaps they were below or above the cell. It cannot be affirmed that they were in the cell and not on the surface, but they seemed as truly in or on the erythrocyte as are malaria plasmodia. This is indicated because they were more frequently found in such situations than chance alone would seem to justify, and especially since all parasites seen in red cells were in the cytoplasm only. Their outlines coincided with the cytoplasmic margin of the host cell, and none of the forms appeared to overlap the chromatin. If their location resulted from chance alone, it is clear that few would occur in situations which so definitely suggested parasitism.

A further interesting observation was apparent schizogony, though no forms were seen in which this process had advanced far. Figs. 3 and 4 show two such parasites, and Figs. 6, 7, and 9 three groups of young forms which suggest origin by schizogony. However, they may also have come from a single disintegrated host cell in which reproduction was by binary fission, and in the liver and spleen no mode of reproduction other than binary fission was observed. It is possible, in view of the occurrence of free forms in the blood, and such forms as those figured which have just been mentioned, that reproduction may occur in the plasma as well as in host cells, either by binary fission or, as just suggested, exceptionally by schizogony.

Although it seemed clear that there was no malaria present, five canaries were inoculated to make doubly sure, and also to test further the possibility that *Toxoplasma* could be transmitted in this way if the dose was heavy enough. One of the five birds was inoculated intravenously with heart blood, two with an emulsion of splenic tissue, and two with a similar emulsion of liver tissue. In all cases the tissue was mixed with isotonic saline citrate solution. After inoculation the birds were followed by making blood smears every day or (after the first ten days) every two or three days. At death each was autopsied and organ smears examined. The results were completely negative as far as the possibility of malaria was concerned.

Of the five inoculated birds, two died within three days of the date of inoculation. The cause of death was uncertain, but it did not appear likely that it was due to *Toxoplasma*, unless in some way the bird was unusually sensitive to the organisms. One of these birds had received a massive injection of liver tissue which was very heavily infected with parasites, and the other received an emulsion of lung. The latter contained a moderate number of parasites, though fewer than the liver.

Two of the other three birds survived for about three weeks, and died apparently of a bacterial infection, presumably acquired from the injection. Both also showed *Toxoplasma*, but the infection was light and could

hardly have been responsible for their death. In one case a single parasite was seen, which in this instance was in the spleen; in the other there were scattered parasites in the lung and liver. The third bird is still alive and apparently in excellent health.

The question arises as to whether the two birds which showed *Toxoplasma* owed their infections to inoculation from the sparrow, or whether they might have already been infected. Herman (1937) reports a series of attempts to infect canaries from sparrows carrying *Toxoplasma*, all of which were negative. Canaries inoculated from sparrows infected both with malaria and *Toxoplasma* in the author's laboratory at various times have never appeared to harbor anything other than the malaria plasmodia afterward. The two birds just referred to above would, however, probably have continued to appear uninfected had autopsies not been made and organ smears examined. There is, therefore, the possibility that in the canary *Toxoplasma* does not ordinarily cause more than a mild, and transitory infection. It is true that Wolfson (1937) reported a number of successful transfers of *Toxoplasma* by inoculation of infected tissue from a canary infected with it to other canaries, and in her experience all successful transfers resulted fatally. Since these birds were also infected with *Plasmodium cathemerium*, however, and since this species exhibits exoerythrocytic schizogony, which stages at that time she regarded as "*Toxoplasma*-like parasites" (Hegner and Wolfson, 1938), it is possible that these infections were not true *Toxoplasma*.

There is another possibility, viz, that canaries are quite commonly infected with *Toxoplasma* when secured from the breeders, or after being kept in the laboratory for a time. Such infection, if mild, would escape notice since it is rather exceptional to see infected cells in the blood, but it might be sufficient to protect the birds against further infection. The evidence for such an assumption is the frequency with which bodies are found in the lungs, sometimes free, and sometimes within mononuclear cells, which bear a considerable resemblance to *Toxoplasma*. Kikuth has referred to them as "Einschlüsse" (Kikuth, 1937; Kikuth and Mudrow, 1938) and the present author has called them "x" bodies (Manwell, 1939). They have the appearance of parasites, and although they do not look exactly like the usual avian type of *Toxoplasma*, they may well be related organisms if not another stage of the same parasite. Kikuth and Mudrow show a colored figure (Fig. 6) and a photomicrograph (Fig. 10) is given by Manwell (1938). Apparently no one has ventured to make any definite suggestion as to the real significance of these bodies, although they have been mentioned by others. They have been seen in both healthy and malarious canaries, and in wild birds.

#### SUMMARY

An infection of toxoplasmosis in a young English sparrow is reported



in which invasion of the erythrocytes was observed on a considerable scale. Since the bird was very young and since subinoculation of blood and organ tissue into canaries failed to reveal any evidence of mixed infection, it is believed that the case was one of infection of *Toxoplasma* alone. The fact that the parasites occurred frequently in the red cells is of considerable interest since it has heretofore been regarded as typically a parasite of mononuclears, and of other types of phagocytic cells. A few forms were seen which were apparently undergoing schizogony, which it has been often thought does not occur in *Toxoplasma*. Two of the five birds inoculated with *Toxoplasma*-infected material (blood in one case, and spleen in the other) showed scattered parasites at autopsy, but died of other causes.

Reasons are suggested for believing that this may not have been a case of genuine transfer, and which may explain the failure of others to secure such transmission.

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## EXPLANATION OF PLATE

FIGS. 1 and 2. Erythrocytes infected with *Toxoplasma*.

FIG. 3. A large macrophage containing what appears to be a schizont.

FIG. 4. A similar schizont free in the blood.

FIG. 5. Numerous free forms such as this were observed in the blood smear. Note the typical crescent-like shape.

FIGS. 6 and 7. Groups of parasites like this were observed in a number of cases in the blood, and suggest origin from schizogony, although they may perhaps have come from a single multiply-infected cell.

FIG. 8. A lymphocyte showing the typical notched nucleus of such cells when infected with *Toxoplasma*.

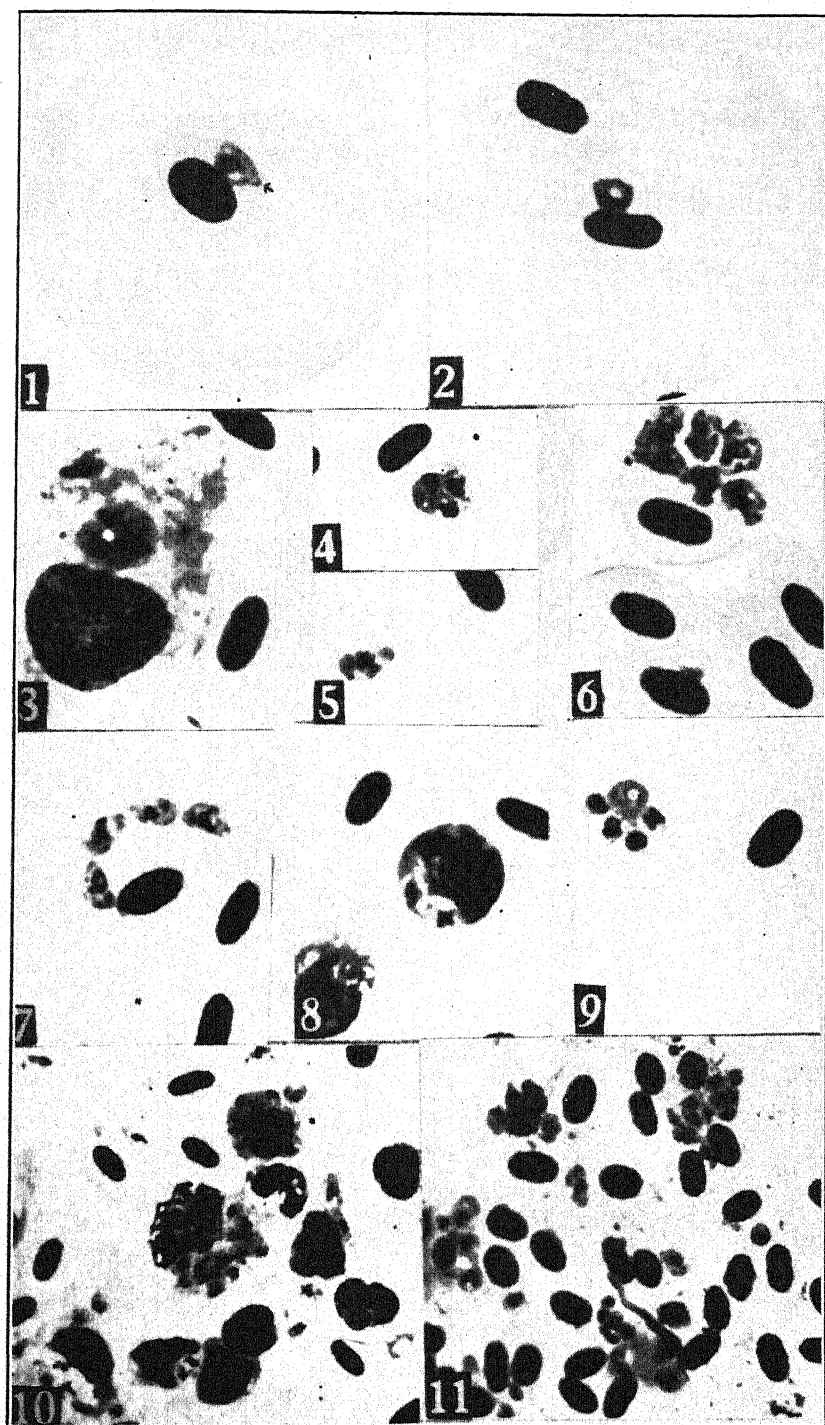
FIG. 9. A group of parasites similar to those shown in Figs. 6 and 7.

FIG. 10. From a liver smear. This figure and the one which follows show how numerous parasites were in these organs.

FIG. 11. From a spleen smear.

(Magnification 2000 $\times$ , except in Figs. 10 and 11, in which it is 1200 $\times$ .)

Microphotographs by Miss Stella Zimmer of the Department of Photography, Syracuse University Medical School.





## THE TREMATODE GENUS *OTODISTOMUM* IN NORTH AMERICA\*

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*Otodistomum* is a genus of digenetic trematodes (AZYGIIDAE) which is widely distributed in elasmobranch fishes. The genus has long been known to occur in rays of both the Atlantic and Pacific coasts of North America, but the status of the species involved has been under discussion for some time. Representative collections from *Raja stabuliformis* from the Isles of Shoals region off the New Hampshire coast and from *Raja binoculata* from Pacific Grove, California, offered opportunity to restudy the characters which have been advanced by earlier workers for recognition of species and varieties of *Otodistomum* in North America.

Stafford proposed the generic name *Otodistomum* in 1904 for trematodes which he identified as *Otodistomum veliporum* (Creplin) from a barn-door skate of Canadian Atlantic waters. Odhner (1911) called attention to the fact that the material which Stafford and several other workers had identified as *O. veliporum* was, according to his interpretation, a distinct species which von Beneden (1871) had named *Distomum cestoides*.

Manter (1926) reviewed the literature and on the basis of a carefully worked out analysis on limited collections from Alaska and Maine concluded that both *O. veliporum* (Creplin) and *O. cestoides* (van Beneden) occur in the North American fauna. After a review of the characteristics of these two species as given in the studies of European investigators, Manter expressed the belief that dimensions of the uterine eggs were about the only significant differences available for separating the two lots of material with which he was dealing, and which he assumed to represent *O. cestoides* and *O. veliporum*. For the Pacific forms, which he identified as *O. veliporum*, he measured 50 eggs finding an average length of 85.5  $\mu$  while his measurement for *O. cestoides* from the Maine coast gave a range of 65 to 72  $\mu$  for egg length with an average of 69.4  $\mu$ . In support of his findings on egg size as a basis for diagnosis he stated (page 41): "This difference is real, constant, and significant. In no case did the egg size overlap between the two species." In the same paper (1926: 57) Manter summarized application of his taxonomic consideration of the two species of *Otodistomum* in the following words: "The egg size is the most certain

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\* Contributions from the Zoological Laboratory of the University of Illinois, No. 580, and the Isles of Shoals Marine Zoological Laboratory of the University of New Hampshire.



distinction between them." In support of Manter's conclusions, stand the earlier observations of Odhner (1911) who found *O. cestoides* eggs 65 to 72  $\mu$  by 43  $\mu$  in diameter and *O. veliporum* eggs about 86  $\mu$  long. Differences of this sort are not available for differentiating the two species in the original specific descriptions. As with so many of the species described by early workers, host and geographical distribution have been relied on to distinguish the two species of *Otodistomum* and to these have been added the details not available to the original describers. Odhner and Dollfus (1937) have given most direct attention to the differentiation of the species and since they agree on host relationships of the species that have been reported from North America, the present writers accept their identification of the specimens from rays of the north Atlantic as *Otodistomum cestoides*.

In this recent critical review of the genus *Otodistomum*, Dollfus accepted Manter's analysis of the dimensions of the egg and Odhner's divergent evidences on thickness of the egg membranes. However, Dollfus did not accept Manter's identifications of the species but concluded that all North American materials represent varieties of the species *Otodistomum cestoides*. For some reason not known to the present writers, Dollfus throughout his paper uses the generic name *Otodistoma*. This is apparently a lapsus for in the original article Stafford (1904) clearly designated *Otodistomum* and this name has been used regularly in the literature since that date.

Relying on Manter's firm belief in egg size as discontinuous between two North American groups, Dollfus sought to add geographical limits to the distribution of varieties on this continent. In his key, he designated *O. cestoides cestoides* as occurring in *Raja* and *Chlamydoselachus* of the north Atlantic and north Pacific (with California included in the latter). He designated *O. cestoides pacificum* as found in *Raja* of the north Pacific and cited the State of Washington and Alaska. The egg measurements he obviously accepted directly from Manter's published observations. However, Dollfus (1937, footnote p. 431) did see material from *Raja binoculata* which had been collected at La Jolla, California (see Sleggs, 1927). Furthermore, he noticed that while eggs which he considered as typical in this California material ranged from 68 to 73  $\mu$  in length by 42 to 43  $\mu$  in breadth, there were other eggs which he classed as atypical which measured only 53 by 34  $\mu$ . Since he accepted Manter's evidence of discontinuity for the varieties which he designated as *O. cestoides cestoides* and *O. cestoides pacificum*, he concluded that both these varieties are to be found in the stomach of *Raja binoculata* which occupies the Pacific coast from Monterey to Sitka.

Linton (1901: 431) had recorded *O. veliporum* from the barn-door skate of the Woods Hole region but in his final summary of his studies

on trematodes from fishes (1940: 107) he corrected the determination to *O. cestoides*, adding several intermediate hosts and *Raja diaphanes* as apparently an accidental host. In his earlier observations, Linton cited 76 by 52  $\mu$  as egg measurements, while in his most recent work he gave 78 by 51  $\mu$ . Realizing that these measurements exceeded those recorded by Manter, Dollfus (still believing in discontinuous ranges in egg size) capitulated with the statement, "il est possible en effet que la longueur des oeufs dépasse un peu 72  $\mu$ ."

An abundance of material of *Otodistomum* was collected from the barn-door skate (*Raja stabuliformis*) at the University of New Hampshire Marine Zoological Laboratory on Appledore Island of the Isles of Shoals near Portsmouth, New Hampshire, in the summer of 1939. For comparison with this material Dr. Harry G. Kimpel had collected a series of well preserved specimens from *Raja binoculata* at the Hopkins Marine Station of Stanford University, located at Pacific Grove, California, during the summer of 1937.

On preliminary examination of the material from California, it was noted that egg sizes were intermediate between the two distinct groups recorded by Manter. In this connection it should be noted that the Alaskan materials studied by Manter were from the same species of host (*Raja binoculata*) as the materials of the present study from Pacific Grove, California. The specimens from this same host from La Jolla, California, which Dollfus recognized as *Otodistomum cestoides cestoides*, are identical with the forms from *Raja stabuliformis* from Maine. Consequently our specimens from *Raja binoculata* from California were submitted to Dr. Manter for identification. In commenting on this material by letter, he mentioned the fact that though the material seemed to be identical with specimens which he had studied from Washington and Alaska, he had noticed that the eggs were smaller in our California material than in specimens at his disposal from farther north on the Pacific coast.

A careful study of a good series of worms in the collection of the senior author has revealed a complete obliteration of the supposed differences between the eggs of what Dollfus has called *O. cestoides cestoides* and *O. cestoides pacificum* as shown in Table 1. In securing information on the eggs, every effort was directed toward securing a fair sample. Ten well oriented eggs in the uterus of each mature worm were measured for length, diameter and thickness of the shell. Of the available mounts from *Raja binoculata* of Pacific Grove, California, 18 were mature, providing measurements for 180 individual eggs. Of the specimens from *Raja stabuliformis* of the Isles of Shoals, New Hampshire, 24 mature worms were used to secure data on 240 individual eggs.

A preliminary analysis of the measurements of entire worms used in

TABLE 1.—Comparisons of American collections of *Otodistomum*

	Body length in mm		Egg measurements in $\mu$					
	Range	Average	Length		Diameter		Shell thickness	
			Range	Average	Range	Average	Range	Average
Host: <i>Raja stabuliforis</i> Manter, 1926 (Maine) . . . .	2.3-65			69.4		46.2		4.5
Linton, 1940 (Woods Hole)	7.5-54			ca 78.0		ca 51.0		3.0
Van Cleave and Vaughn (Isles of Shoals)	6.9-30.2	21.14	55-90	72.6	36-58	47.2	3-4	3.6
Host: <i>Raja binoculata</i> Manter, 1926 (Alaska) . . . .				85.5		57.8		4.0
Dollfus, 1937 (La Jolla) . . . .	ca 61		53-73		34-49		2-5	
Van Cleave and Vaughn (Pacific Grove) . . . . .	23.9-69	43.98	60-94	77.2	40-70	52	3-5	3.67

this study seemed to indicate that while forms from the Pacific averaged longer than those from the Atlantic there was considerable overlapping in the ranges of the lengths. Further evidence of the instability of body length as a criterion for distinguishing varieties of *Otodistomum* is provided by the fact that Manter secured series of worms from *Raja stabuliforis* off the Maine coast, but a short distance from the area from which our specimens were taken from the same host species, and his worms were distinctly larger than the ones which we studied. It may be that the length of the growing period in an alternate host influences the size of the mature trematodes in the definitive host. Additional evidence of individual variation in size is provided by the size at which individuals reach sexual maturity. Manter states that "sexual maturity is attained between body lengths of 10 and 15 mm" but in our collection from the Atlantic coast one individual 6.9 mm long had the uterus filled with eggs while two individuals 6.1 and 7.6 respectively had no eggs. Size at which individuals of *Otodistomum* reach maturity shows great individual variability.

In an attempt to see if any correlation might exist between relative size of the mature worm and size of the eggs it contained, it was noticed that egg size was variable in all individuals regardless of the total length of the worm and that both large and small eggs appeared without distinct segregation in animals of the various lengths.

In an effort to find morphological features for distinguishing two species of *Otodistomum* in North American hosts, Manter reviewed the literature and made a careful search, but concluded that the two forms from the Atlantic and the Pacific could be separated only on the basis of

discontinuous ranges of egg sizes. Since the present series of observations on new materials from both coasts of North America show complete intergradation in egg size, this criterion no longer holds as the basis for separating two subspecies as proposed by Dollfus. Consequently, the present writers maintain that the American forms comprise a single highly variable species to which the name *Otodistomum cestoides* should be applied.

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## THE SLENDER LICE OF AMERICAN PIGEONS AND DOVES WITH DESCRIPTIONS OF TWO NEW SPECIES

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The American Ornithological Union Checklist gives 21 species and subspecies of pigeons and doves. Of these, only six are found in large numbers over any considerable areas north of Mexico. The writer had hoped to secure lice from at least these six before studying the group critically. However the past fifteen years have brought in lice from only five different kinds of pigeons and doves. It is hoped that the present report may help to focus attention on the parasites of these birds so that lice from the other species and subspecies will be collected.

The slender lice of pigeons and doves at hand includes *Esthiopterum columbae* (Linn.) from the domestic pigeon and *Esthiopterum baculoides* (Paine) from the western mourning dove. Comparisons of these with lice from the eastern mourning dove, eastern ground dove and the band-tailed pigeon show that we must add two new species to those previously recognized.

Ewing erected the genus *Columbicola* for the slender lice of doves and pigeons in 1929 almost solely on the basis of two pairs of spines on the dorsal surface of the clypeus of *Esthiopterum columbae*. He describes an anterior and a posterior pair. These spines are present on the four species to be discussed. However, Malcomson (1937) in describing *Columbicola extincta* from the passenger pigeon states that the recurved setae (posterior spines) are not present. Until more lice from pigeons and doves can be critically studied to establish a more complete characterization of the group, the writer is considering *Columbicola* as a subgenus.

### *Esthiopterum* (*Columbicola*) *passerinae* n. sp.

(Figs. 1-4)

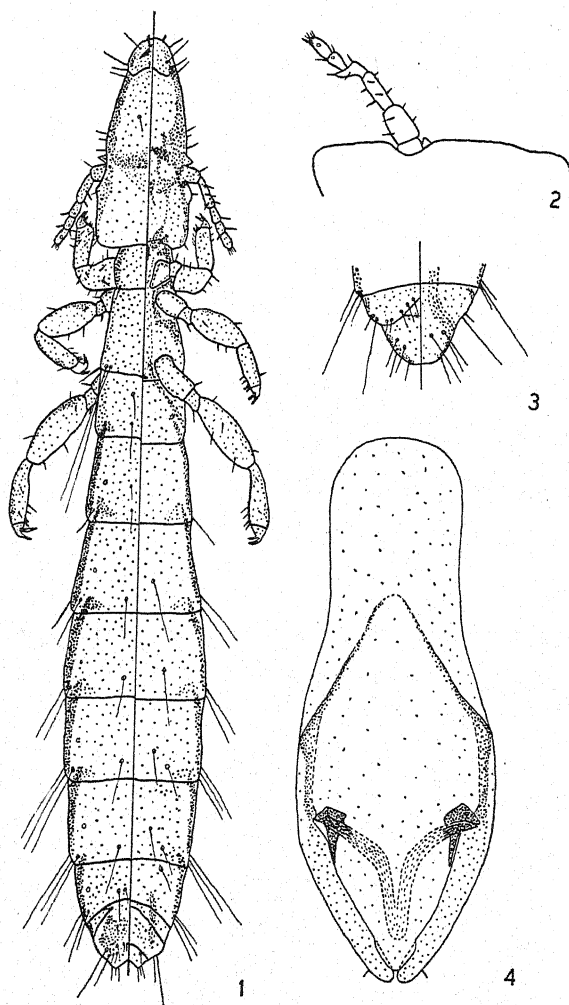
*Female*, Fig. 1. Body form and chaetotaxy as shown by Fig. 1, the outline of which is a tracing of a microprojection of the allotype. Head length of this specimen is .528 mm; head width across temples .251 mm. From these measurements other dimensions may be computed from the Fig.

*Male*, Figs. 2, 3 and 4. Resembles the female in body form and chaetotaxy. Is slightly smaller. Third segment of antenna with thumb-like projection. Terminal segments of abdomen differ from female as shown by comparisons of Figs. 1 and 3. Following head measurements (in mm) are indicative of size and variation in the specimens available.

Head length .....	.489	.475	.482	.489	.475	.489	.475
Head width .....	.251	.238	.238	.251	.238	.238	.224

Male genitalia, Fig. 4, consists of a basal plate to which the parameres appear to be immovably joined. Endomeres are distinct, separate and short. Preputal sac

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FIGS. 1-4. *Esthiopterum (Columbicola) passerinae* n. sp.

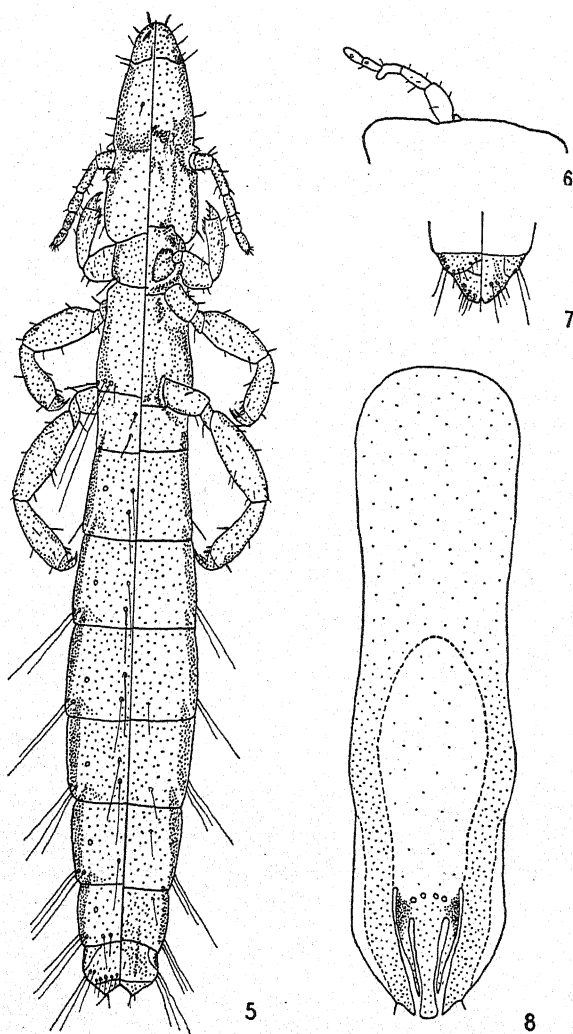
FIG. 1. Female; FIG. 2. Outline of head and antenna of male; FIG. 3. Posterior end of abdomen of male; FIG. 4. Ventral view of male genitalia.

or vesicula penis with a characteristic V-shaped thickening, the arms of which extend anteriorly to the endomeres and appear to articulate with them.

*Host*: Eastern ground dove, *Columbagallina passerina passerina* (Linn.): 6 males and 1 female collected by H. S. Peters at Auburn, Ala., April 4, 1936; 3 males collected at Slocumb, Ala., by A. G. Watkins, Jan. 4, 1937.

*Types*: The holotype male and the allotype are deposited in the Cornell University Collection. Paratypes are deposited in the following entomological collections: U. S. Nat. Mus., Washington, D. C.; Canadian National Collections, Ottawa, Canada; British Museum of Natural History, London, England; Stanford University, Calif.; Univ. of Minnesota, St. Paul, Minn.; Tulane University, New Orleans, La.

This species is characterized by its small size and male genitalia. The posterior clypeal spines are thickest just above the base whereas in the other species to be mentioned with the exception of *E. macrourae* they taper evenly from the base. The posterior clypeal spines are markedly thicker than any part of the anterior clypeal spines and longer. This is in contrast to *E. baculoides* (Paine) in which the posterior clypeal spines are less thick than even the basal part of the anterior clypeal spines and both pairs of spines are very slender and hair-like and about equal in length.



FIGS. 5-8. *Esthiopterum (Columbicola) macrourae* n. sp.

FIG. 5. Female; FIG. 6. Outline of head and antenna of male; FIG. 7. Posterior end of abdomen of male; FIG. 8. Ventral view of male genitalia.

*Esthiopterum (Columbicola) macrourae* n. sp.  
(Figs. 5-8)

*Female*, Fig. 5. Body form and chaetotaxy as shown by Fig. 5, the outline of which is a tracing of a microprojection of the allotype. Head length of this specimen is .620 mm; head width across temples is .264 mm. From these measurements other dimensions may be computed from Fig. 5. Following head measurements (in mm) are indicative of size and variability:

Head length	....	.620	.620	.620	.620	.634	.607	.620	.607
Head width	.....	.277	.290	.277	.264	.277	.264	.264	.277

*Male*, Figs. 6, 7 and 8. Resembles the female in body form and chaetotaxy. Is somewhat smaller. Third segment of antenna with thumb-like projection. Terminal segments of abdomen differ from female as shown by comparison of Figs. 7 and 5. Following head measurements (in mm) are indicative of size and variation:

Head length	....	.581	.581	.594	.554	.568	.581	.555	.554
Head width	.....	.251	.264	.264	.238	.251	.264	.251	.264

Male genitalia, Fig. 8, consists of a basal plate to which the parameres are apparently fused. They have almost transparent lateral borders. Endomeres are elongated with thickened basal regions, extending medially and with mesal borders difficult to delineate.

*Host*: Eastern mourning dove, *Zenaidura macroura carolinensis* (Linn.): 4 males, 21 females and several young collected by T. F. Hall, Jr. Dec. 2, 1934; 8 males collected by Ernest Beck Dec. 2, 1934; 5 males and 8 females collected by Ernest Beck Dec. 21, 1934. All were collected near New Orleans, La.

*Types*: The holotype male and the allotype are deposited in the Cornell University Collection. Paratypes are deposited in the following entomological collections: U. S. Nat. Mus., Washington, D. C.; Canadian National Collections, Ottawa,

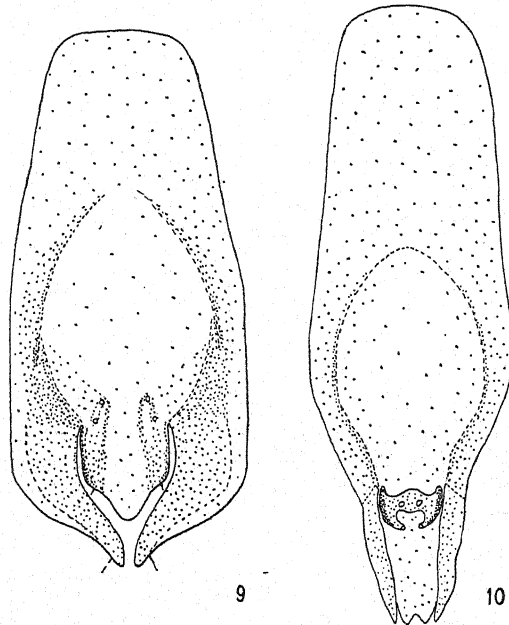


FIG. 9. Ventral view of male genitalia of *Esthiopterum (Columbicola) columbae* (Linn.).

FIG. 10. Ventral view of male genitalia of *Esthiopterum (Columbicola) baculoides* (Paine).

Canada; British Museum of Natural History, London, England; Stanford University, California; Univ. of Minnesota, St. Paul, Minn.; Herbert Osborn's Collection at Ohio State University, Columbus, Ohio; Tulane University, New Orleans, La.

This species is characterized by its head being longer than that of any of the other species mentioned, its relatively narrow temples, and the male genitalia. The male genitalia of *E. columbae*, Fig. 9, is given for comparison. The clypeal spines resemble *E. passerinae* but with a less noticeable enlargement near the base of each posterior spine. Anterior and posterior spines about equal in length.

The slender louse of the eastern mourning dove has been reported by previous workers (Peters, 1933; Geist, 1931) as *Esthiopterum columbae* (Linn.) or *Columbicola columbae* (Linn.). Emerson (1940) reports *Columbicola columbae* from a mourning dove without mentioning the variety of mourning dove.

It is expected that a critical examination of the specimens on which these reports were made would show them to be *E. macrourae*, except possibly the Oklahoma record. The eastern and western varieties of mourning dove are both found in Oklahoma.

#### DISCUSSION

A peculiar host specificity is exemplified by these lice on pigeons and doves. *E. columbae* seems to be restricted to the domestic pigeon. The report of *Lipeurus baculus* Nitzsch, a synonym of *E. columbae* (Linn.) on *Columbagallina passerina socorroensis* Kellogg and Mann, 1912, is probably an erroneous identification. It had been expected that the slender louse of the eastern and western mourning doves would be identical. Paine (1910) described *E. baculoides* from the western mourning dove. In this description he mentioned the absence of a thumb-like process on the third antennal segment of the male. The absence of this process mentioned in the description was questioned until the examination of a co-type loaned by the Stanford University Museum through the courtesy of Professor G. F. Ferris, showed the actual absence of this thumb-like appendage. The absence of this appendage was further checked by an examination of over 35 males collected from the western mourning dove at Pasadena, California, Aug. 21, 1937, by S. F. Wood. Paine's description checks in all details. Paine did not describe or figure in detail the male genitalia of *E. baculoides*, so, in order to complete the evidence for the separation of these two species, the male genitalia of Paine's species is illustrated in Fig. 10. The host relationships are further complicated by the fact that ten male and eighteen female lice, collected at Ojo de agua, Galeano, N. L. Mexico, Aug. 15, 1938, by H. Hoogstraal from *Columba fasciata fasciata* are indistinguishable from the new *Esthiopterum macrourae* from the eastern mourning dove except for the clypeal spines which are like those of *E. columbae*.



A comparative study of the male genitalia of the four species mentioned above is particularly interesting. The parameres of all are specialized in that they are immovably joined to the basal plate. The position of other parts points definitely to the place of fusion, and *E. baculoides* shows a thin line only, separating the parameres from the basal plate. This thin line indicates the place of fusion rather than a functional joint. The endomeres are more variable, showing as two separate and distinct structures in *E. passerinae* from the ground dove, and as separate, poorly delineated structures fused to the sides of the preputal sac or vesicula penis in *E. columbae*. In *E. macrourae* from the eastern mourning dove and from the banded pigeon, they are elongate, showing a mesal development. In *E. baculoides* they have grown together medially and form a distinctive endomeral plate.

*Columbicola extincta* was described by Malcomson (1937) from the passenger pigeon but the drawing of the male genitalia is not sufficiently clear to include it in the above comparisons.

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## OBSERVATIONS ON THE VIABILITY OF EGGS OF LUNGWORMS OF SWINE\*

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Little information is available concerning the survival period of eggs of swine lungworms on and in soil and the effects on these eggs of various environmental factors. An investigation was undertaken, therefore, to ascertain (1) the survival time of lungworm eggs in feces located on or beneath the surface of soil outdoors; (2) the effect on the survival period of the eggs of vegetation growing on the infested soil; and (3) the effect on the eggs of high and low temperatures and drying. It was considered that information of this nature could be used to improve current recommendations for the control of lungworms in swine.

### EXPERIMENTAL PROCEDURE

In the tests herein described eggs of two species of lungworms, *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*, contained in feces of naturally infected pigs were used. The measure of the viability of the eggs was the ability of the contained larvae to infect susceptible earthworms (*Helodrilus* species). Tests of the viability of eggs were carried out as follows:

Feces containing the eggs were first thoroughly mixed with moist, helminthologically-sterile soil in the proportion of approximately 14 grams of feces to 100 grams of soil. The mixture of soil and feces was then placed in finger bowls and six to eight earthworms were added to each container. The soil-feces mixture was covered with moist absorbent paper and the dishes were loosely covered to prevent excessive evaporation of moisture; the cultures were maintained at a temperature of 20° to 25° C for periods of 21 to 30 days. The earthworms from the cultures were then dissected, and the number of lungworm larvae present in the hearts and anterior portion of the digestive tract of each annelid was counted. The localization of the larvae in the latter region was described by Schwartz and Porter (1938). In cases where the larvae were massed in the hearts, the number was estimated as accurately as possible.

In the tests of the longevity of lungworm eggs on and in soil under outdoor conditions, 2,000 grams of feces, containing approximately 1,000 eggs per gram, were placed in each of eight unshaded plots prepared as follows:

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\* This investigation was carried out from June, 1938, to December, 1939, at the U. S. Department of Agriculture Beltsville Research Center, Beltsville, Maryland.

- Plot 1—Feces on top of soil kept free of vegetation  
 Plot 2—Feces on top of soil planted to barley  
 Plot 3—Feces 4 inches beneath surface kept free of vegetation  
 Plot 4—Feces 4 inches beneath surface planted to barley  
 Plot 5—Feces 6 inches beneath surface kept free of vegetation  
 Plot 6—Feces 6 inches beneath surface planted to barley  
 Plot 7—Feces 12 inches beneath surface kept free of vegetation  
 Plot 8—Feces 12 inches beneath surface planted to barley

When the vegetation on plots 2, 4, 6 and 8 died, these plots were reseeded. At intervals indicated in table 1 enough fecal material to fill

TABLE 1.—Time lungworm eggs in feces on and in soil survived as determined by earthworm feeding tests on exposed eggs

Eggs on and in bare (unseeded) soil					
Plot number	Location of eggs	Date sampled	Duration of exposure	Earthworms examined	
				Number	Larvae recovered*
			<i>Days</i>		<i>Number</i>
1	On surface	August 10, 1938	32	8	Less than 1
1	do	August 31, 1938	43	5	Less than 1
3	Buried 4 inches	do	43	1	21
5	Buried 6 inches	do	43	5	25
7	Buried 12 inches	do	43	3	182
1	On surface	February 2, 1939	198	4	0
3	Buried 4 inches	do	198	3	19
5	Buried 6 inches	do	198	3	26
7	Buried 12 inches	do	198	3	114
1	On surface	May 5, 1939	290	3	Less than 1
3	Buried 4 inches	do	290	3	11
5	Buried 6 inches	do	290	3	26
7	Buried 12 inches	do	290	3	55
1	On surface	November 9, 1939	381	3	0
3	Buried 4 inches	do	381	3	Less than 1
5	Buried 6 inches	do	381	4	1
7	Buried 12 inches	do	381	3	2
Eggs on and in grassy (seeded) soil					
2	On surface	August 10, 1938	32	4	1
2	do	August 31, 1938	43	7	0
4	Buried 4 inches	do	43	3	133
6	Buried 6 inches	do	43	5	105
8	Buried 12 inches	do	43	3	32
2	On surface	February 2, 1939	198	3	0
4	Buried 4 inches	do	198	3	38
6	Buried 6 inches	do	198	3	82
8	Buried 12 inches	do	198	3	105
2	On surface	May 5, 1939	290	3	1
4	Buried 4 inches	do	290	3	91
6	Buried 6 inches	do	290	3	114
8	Buried 12 inches	do	290	3	160
2	On surface	November 9, 1939	381	3	0
4	Buried 4 inches	do	381	4	7
6	Buried 6 inches	do	381	4	3
8	Buried 12 inches	do	381	4	0

\* Average per earthworm.

without packing a 50 cc bottle was collected from each plot and the viability of the eggs ascertained by earthworm feeding tests. The sampling of the plots was terminated after 381 days as the fecal material in the plots had been largely exhausted by the sampling.

Tests involving the effect of low temperature on the eggs were con-

ducted with 14-gram samples of feces in tightly closed sputum jars subjected continuously to temperatures from  $-8^{\circ}$  to  $-20^{\circ}$  C or lower for periods from 3 to 108 days. At intervals (see Table 2) samples were

TABLE 2.—*Effect of continuous freezing at temperatures of  $-8^{\circ}$  to  $-20^{\circ}$  C on viability of lungworm eggs as determined by earthworm feeding tests*

Sample number	Period of exposure	Temperature of sample at removal	Earthworms examined	
			Number	Larvae recovered*
	Days	$^{\circ}$ C		Number
1	3	$-11$	3	230
2	7	$-8$	4	204
3	10	$-10$	3	219
4	14	$-10$	2	223
5	21	$-12$	3	279
6	24	$-14$	4	277
7	27	$-12$	3	188
8	38	$-10$	6	244
9	42	$-14$	4	323
10	46	$-8$	3	343
11	49	$-8$	3	142
12	63	$-20$	8	91
13	87	$-10$	5	99
14	108	$-14$	3	8
Control	108	$2^{\circ}$ C entire period	5	319

\*Average per earthworm.

removed, allowed to thaw at room temperature, and the viability of the eggs tested. A control sample maintained at a temperature of approximately  $2^{\circ}$  C was tested for viable eggs at the same time as the last test sample.

In the tests involving the effect of desiccation on eggs, 5 grams of feces were evenly spread on each of several 3-inch square glass plates, and the feces partially dried in a strong current of air; the plates were then kept over calcium chloride in a partial vacuum for an additional period of approximately 6 hours to insure complete drying of the fecal material. Certain of these dried preparations were then subjected for varying periods to a continuous temperature of  $17^{\circ}$  to  $22^{\circ}$  C whereas others were subjected to a temperature of  $37^{\circ}$  C and still others to a temperature of  $1^{\circ}$  to  $2^{\circ}$  C. Control cultures were not subjected to desiccation.

Tests of the effect of high temperature on the eggs were carried out as follows:

Approximately 14 grams of moist feces containing eggs were tightly packed in each of a series of sputum jars that were tightly stoppered with corks each containing a thermometer for recording the temperature of the feces, and a capillary tube to permit escape of gases from the fecal material. The test jars were then immersed in a water bath held at the desired temperature. In some cases the jars were removed as soon as the temperature of the feces became equal to that of the water; temperatures of  $40^{\circ}$ ,  $50^{\circ}$ ,  $55^{\circ}$ ,  $60^{\circ}$ ,  $65^{\circ}$ , and  $70^{\circ}$  C were employed. In an-

other test, the jars were allowed to remain in the water bath for 10 minutes after the temperature of the feces had become equal to that of the water; temperatures of 40°, 50°, 55° to 60°, and 65° to 70° C were employed. In all cases when the jars were removed from the water bath they were immediately immersed in ice water until the temperature of the feces was approximately 30° C; the viability of the eggs was then tested by earthworm feeding. Control cultures were kept at a temperature of 25° to 30° C and were not subjected to higher temperatures.

#### EXPERIMENTAL RESULTS

The major results of these tests are summarized in Tables 1 to 3. The following brief presentation of the data will serve to point out some of the more outstanding facts elicited, and present certain data not summarized in tables.

##### *Length of Survival of Eggs on and in Soil of Bare and Grassy Plots*

The data summarized in Table 1 show that although the viability of the majority of eggs in feces located on the surface of plots 1 and 2 was destroyed in 32 days or less, a small number retained their viability as long as 290 days. As can be seen from the data, there was no significant difference either in rate of death of eggs or in maximum length of survival of eggs between the seeded and unseeded plots. It may be concluded from this, therefore, that under the condition of the test the moderately heavy growth of vegetation present on plot 2 did not materially affect the time of survival of the eggs.

In contrast to this the rate of death of eggs kept buried at varying depths beneath the surface (plots 3 to 8, inclusive) was noticeably less rapid during the first 290 days of the test. At this time the average number of larvae recovered from earthworms fed eggs from the surface plots 1 and 2 was one or less, whereas average infections of 11 to 160 larvae occurred in earthworms fed eggs buried to varying depths in plots 3 to 8. Subsequent to the first 290 days of the test the buried eggs may have died rapidly as only slight infections resulted from earthworm feedings 381 days after the beginning of the test (Table 1). However, this may have been due in part to depletion of the fecal material in the plots as a result of the sampling procedure, since only relatively small amounts of the fecal material could be located at the time the last samples were taken.

As adjudged by the results of the earthworm feeding tests carried out at 43, 198, and 290 day intervals on eggs in feces that had been kept buried 4 and 6 inches deep in plots 3 to 6, inclusive, the rate of death of eggs in the seeded plots was noticeably less rapid than in the unseeded plots. For example, in the first three feeding tests the average numbers



of larvae recovered from earthworms fed eggs from unseeded plots numbers 3 and 5 varied from 11 to 26, whereas the average infections in earthworms fed eggs from seeded plots numbers 4 and 6 varied from 38 to 133 (Table 1). In the case of the seeded plots numbers 4 and 6 the grass roots had penetrated into the fecal material rendering it less compact, and perhaps at the same time kept the moisture content more favorable for the survival of the eggs than was the condition in the unseeded plots.

In the case of eggs in feces buried 12 inches in plots 7 and 8, a distance too great for the grass roots to penetrate, no consistent difference was noted between the average infections of earthworms fed eggs from these plots at intervals throughout the test (Table 1). It is interesting to note, however, that during the first 290 days of the test the rate of death of eggs buried 12 inches was apparently less rapid than that of eggs buried 4 and 6 inches as adjudged by results of earthworm feeding tests. That is, larger numbers of lungworm larvae were recovered in the earthworm feeding tests of the viability of eggs in plots 7 and 8, than in the case of plots 3 to 6 inclusive. However, as stated previously, in the final sampling of plots 7 and 8 (November 9, 1939), only small amounts of the buried fecal material could be located, which may account in part for the very small number of larvae recovered in these tests.

#### *Effect of Continued Low Temperatures on Eggs*

As shown by the results of earthworm feeding tests recorded in Table 2, some lungworm eggs in feces were able to survive continuous freezing at temperatures of  $-8^{\circ}$  to  $-20^{\circ}$  C or lower as long as 108 days. During the first 49 days of the test, when the temperatures varied from  $-8^{\circ}$  to  $-14^{\circ}$  C, there was apparently no appreciable effect on the eggs as shown by the fact that the number of larvae recovered from earthworms in feeding tests carried out did not materially decrease during that period. During the period from the 56th to the 63rd day of the test the temperature of the freezing unit employed varied from  $-20^{\circ}$  to  $-27^{\circ}$  C, and during the entire 62nd day remained at  $-40^{\circ}$  C; this may have accounted for death of some of the eggs shown by the small numbers of larvae recovered from the test earthworms during this period and later.

That the reduction in numbers of larvae recovered over this test period was due to the inimical effects of the low temperature is indicated by the fact that 319 larvae (average) were recovered per earthworm from a control culture of feces kept 108 days at  $2^{\circ}$  C.

#### *Effect of Continuous Desiccation on Eggs*

As can be seen from the data presented in Table 3, although a small proportion of lungworm eggs contained in feces dried on glass was able

TABLE 3.—*Effect of desiccation at 1° to 2° C and 37° to 39° C on the viability of lungworm eggs as determined by earthworm feeding tests*

Test number	Period exposed	Temperature	Earthworms examined	
			Number	Larvae recovered*
	<i>Days</i>	<i>°C</i>		<i>Number</i>
1	1	17 to 22	3	19
2	3	do	4	8
3	4	do	2	4
4	6	do	3	4
5	7	do	5	10
Control	None	do	5	319
6	10	37	4	3
7	10	17 to 22	3	20
8	10	1 to 2	3	61
Control	None	17 to 22	1	295
9	25	37 to 39	3	0
10	25	17 to 22	3	0
11	25	1 to 2	3	37
Control	None	17 to 22	2	727
12	31	37 to 39	3	0
13	31	17 to 22	3	0
14	31	1 to 2	3	14
Control	None	17 to 22	3	422
15	38	37 to 39	3	0
16	38	17 to 22	3	0
17	38	1 to 2	3	30
Control	None	17 to 22	3	422

\* Average per earthworm.

to survive 38 days continuous desiccation at a temperature of 1° to 2° C, none was able to survive 25 days or longer at a temperature of 17° to 22° C or higher. Moreover, continued desiccation of only 7 days at a temperature of 17° to 22° C under the conditions of the test was apparently lethal to the majority of eggs as shown by the fact that an average of only 10 larvae was recovered from earthworms fed eggs dried under these conditions as compared to an average of 319 larvae acquired by earthworms from control cultures (Table 3). These observations explain in part failure of the majority of lungworm eggs on soil to survive more than 32 days in late summer tests herein reported (Table 1).

#### *Effect of High Temperatures on Eggs*

In a series of tests in which the temperature of small quantities of moist feces containing lungworm eggs was raised rapidly to 60° C and then quickly lowered to about 30° C, small numbers of eggs survived and were able to infect susceptible earthworms. However, in a later series of tests no eggs survived temperatures of 55° C or higher.

In order to determine the thermal death point of the eggs, a series of cultures was subjected to temperatures of 40°, 50°, 55° to 60°, and 65° to 70° for 10 minutes each. As determined by earthworm feeding tests, small numbers of eggs survived temperatures of 40° and 50° C but none survived 55° C or higher.

#### DISCUSSION

Data herein reported with regard to the marked resistance of lung-

worm eggs to continued low temperatures and their long survival in soil under favorable moisture conditions serve to explain in part the occurrence of lungworm-infected earthworms in soil of areas unused by hogs for as long as 4 years (Schwartz and Alicata, 1934; Spindler, 1938). The facts set forth in this paper have a bearing on the control of lungworm infections in pigs by management practices. Since as shown in the data lungworm eggs are most susceptible to desiccation and high temperatures, raising pigs on well-drained temporary pastures where lungworm eggs would be exposed to the inimical effects of dryness and the high temperatures engendered by the sun may be the most effective of the control measures against this parasite. In view of the long period of survival of lungworm eggs herein reported, plowing under of soil contaminated with lungworm eggs and growing a crop thereon might not be effective in hastening the destruction of the eggs but might on the contrary favor their survival. In light of the data presented in this paper, permitting an area to lie fallow for a period of at least a month during summer following removal of lungworm-infected animals should hasten destruction of lungworm eggs by desiccation and high temperatures. Subsequent plowing would tend to scatter the remaining viable eggs to such an extent in the soil that earthworm infections would be reduced to a minimum. Such measures might aid materially in controlling lungworm infections in pigs subsequently maintained on the areas in question.

#### SUMMARY

A series of tests designed to ascertain (1) the survival time of eggs of swine lungworms (*Metastrongylus elongatus* and *Choerostrongylus pudendotectus*) on and in soil, (2) the effects of low and high temperatures, and of drying on these eggs were carried out at Beltsville, Md.

The viability of eggs in feces on the surface of unshaded outdoor plots was destroyed in about 25 days. Some eggs in feces buried 6, 8, and 12 inches survived 381 days, but most of these eggs succumbed in about 290 days.

Under laboratory conditions the viability of eggs in moist feces was destroyed by short exposure to a temperature of 55° C. Eggs in moist feces survived 108 days at a temperature of -8° C to -20° C.

Eggs in dried feces were destroyed in 25 days at a temperature of 17° to 22° C. Eggs in dried feces at a temperature of 1° to 2° C were still viable after 38 days.

Information contained in this paper suggests that permitting soil contaminated with swine lungworm eggs to lie fallow about a month before plowing should be useful in controlling these parasites.

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## REVIEWS

AN INTRODUCTION TO NEMATODOLOGY. By B. G. Chitwood and M. B. Chitwood, with the collaboration of A. C. Walton, Reed O. Christenson, Leon Jacobs and F. G. Wallace. Section I, Part III, and Section II, Part I: pp. 125-240, Figs. 112-164. Published by the authors, Babylon, N. Y. 1940. \$4.50.

This publication is the concluding portion (Part III) of Section I and the first part of Section II of a comprehensive treatise on nematology. As was to have been expected, the authors have produced an excellent companion piece for the preceding portions, the first of which appeared in 1937, the second in 1938. Section I, Part III, contains five chapters (IX-XIII). The first three are concerned with the excretory system, the reproductive system, and the nervous system. In them, the fundamental patterns and variations which characterize these systems in various nema groups are skillfully analyzed. Collation of the extensive literature on these subjects, to which the authors have contributed significantly themselves, has resulted in a product for which the authors deserve much praise.

Chapter XII, prepared by R. O. Christenson with the collaboration of Leon Jacobs and F. G. Wallace, is entitled "Nemic Ova" and deals primarily with the morphological variations encountered in the eggs of a wide variety of nemas. Attention is also devoted to the chemical character of the egg membranes. The chapter contains two excellent full page plates of drawings illustrative of the various types of eggs from representative nemas. No student of parasitology should deny himself the examination of these figures, or for that matter, possession of the "Introduction."

To those with zoological backgrounds, the last chapter (XIII) of Section I will prove interesting. Dealing as it does with the controversial subject of "Nemic Relationships," one would expect sufficient latitude for considerable disagreement. The author, B. G. C., has been conservative and competent in dealing with zoological groups outside his special field. A careful reading of the chapter impresses one that he has also recognized the limitations inherent in the subject and has striven to be objective concerning the tangible characters which must be evaluated in reaching any conclusions regarding relationships. Differences with the author's conclusions which may ensue, will arise primarily from a lack of agreement on the fundamental characters to be considered and the assessment of their value for the end in view. The historical aspect of the subject has been handled well and is interesting reading. The author's tabulation and evaluation of characters in various groups is ingenious and deserves critical study.

The concluding three chapters are in Section II, Part I. The first, by A. C. Walton, is a concise résumé of extant knowledge on gametogenesis, meiosis and fertilization. A table of chromosome numbers and other pertinent information covering 50 different nematodes is included. Chapter II deals with "Nemic Embryology" and covers the principal aspects of the determinate cleavage and constancy of cell numbers which characterizes the development of these animals. The final chapter is on postembryonic development. Numerous variations in the development of nematodes are recorded and several hiatuses in existing knowledge on the subject indicated.

In keeping with the precedent established for the previously issued parts of this treatise, the present part is profusely illustrated and each chapter concludes with an extensive bibliography. The compilation and integration of the information on nematodes which is represented in this part, as well as in the preceding parts, was a monumental undertaking. The Chitwoods are to be congratulated on the success they have achieved. It is not inappropriate to refer to the data recorded on the last page. Clearly, the authors merit more financial support for this personal venture than they seem to be getting from zoologists, parasitologists and nematologists generally.



It is necessary to call attention to the fact that the printing is inferior in quality in comparison with that of the previous parts. Whether the economy achieved was worth the price is debatable. It is likewise unpleasant to note that the proof was not corrected with the care which the reviewer believes the publication merited: a list of 70 errata, typographical and otherwise, observed by the reviewer in 115 pages seems remarkably long. Perhaps one should not notice mediocrity on this point when the substance is so fine.—GEORGE L. GRAHAM.

CLINICAL PARASITOLOGY. By Charles Franklin Craig and Ernest Carroll Faust. 772 pages, 244 illustrations. Lea and Febiger, Philadelphia. 1940. 2nd edition. \$8.50.

The necessity for bringing out a second edition of this work within three years after the first (reviewed, 1938, *J. Parasitol.* 24: 94) attests the cordial reception that it has received. In preparing the new edition the authors have taken full advantage of the opportunity to revise the original text and to include recent additions to knowledge in the subject. The present volume contains 39 more pages and several new illustrations. The most notable addition is a new chapter on leeches of medical importance. The technical appendix has been enlarged to include some of the more recent diagnostic procedures such as the zinc sulfate flotation technic and the NIH anal swab technic of examining for pinworms. The many recent contributions to knowledge of tick-borne diseases and the epidemiology of yellow fever and plague are adequately covered. Since the text is so up to date in other respects, it is to be regretted, however, that more adequate discussion is not given to the latest work on immunity in experimental helminth infections.

The new edition maintains the high standard of typography and excellence of printing established by the first. Few errors were detected. On page 273, however, the amount of blood withdrawn per day by a hookworm in the intestine is erroneously given as 0.1 cu mm, which would be a negligible amount.

An extensive bibliography, together with a complete author index, greatly enhances the value of the book for reference. The work is to be highly recommended to graduate students in parasitology and also to medical students and practitioners who wish to have a complete, authoritative and up-to-date text on the subject.—O. R. McCoy.

A GUIDE TO HUMAN PARASITOLOGY FOR MEDICAL PRACTITIONERS. By D. B. Blacklock and T. Southwell. 259 pages, 122 illustrations and 2 colored plates. Williams and Wilkins, Baltimore. 1940. 4th edition. \$4.00.

The present edition of this work, which is intended primarily as a manual for beginners interested in human parasitology, is essentially unchanged from the last edition issued in 1938 (reviewed, 1939, *J. Parasitol.* 25: 515). The only changes discovered are a slight revision of the table of the life cycle of *Strongyloides* (page 171), the addition of several footnotes and the correction of misprints. Because of the simplified presentation, the book will continue to be of value to the medical practitioner or public health worker with a superficial interest in the subject. The numerous epidemiological tables and charts of life histories are particularly valuable in this respect.

With the exception of brief mention of myiasis in a chapter of miscellany, medical entomology is not included in the subject matter. A short chapter is devoted, however, to the spirochaetes, although it is recognized that this group is more closely related to the bacteria than to the protozoa. In view of the space devoted to some of the rarer helminth parasites, it would seem that the intestinal flagellate group is deserving of more extensive consideration. For instance, *Trichomonas vaginalis* is not mentioned anywhere in the text, in spite of the fact that this parasite is recognized to occur commonly in all parts of the world and the question of its pathogenicity is widely debated in gynecological literature. The reviewer would also suggest that the subject matter in the latter chapters of the book could be more logically arranged. Since the terminology is on the whole up

to date, it is to be regretted that "*Taenia granulosa*" is used instead of *Echinococcus granulosus*, the name approved by the International Commission on Zoological Nomenclature.

This work is to be recommended particularly to those who desire a concise aid for the diagnosis of human parasites. Also, it may well be used as a text-book by medical students who receive only brief training in the subject of parasitology.—O. R. McCoy.

1940 YEAR BOOK OF PUBLIC HEALTH. Edited by J. C. Geiger. 560 pages. The Year Book Publishers, Inc., 304 South Dearborn Street, Chicago. \$3.00, postpaid.

This volume by Dr. Geiger (Director of Public Health, San Francisco) attempts to survey the advances in the field of public health. The following subjects are considered:

1. Communicable Diseases and Epidemiology: 1a, General; 1b, Tuberculosis; 1c, Poliomyelitis; 1d, Malaria; 1e, Venereal Diseases. 2. Food and Milk. 3. Nutrition. 4. Housing. 5. Statistical. 6. Laboratory. 7. Industrial Hygiene. 8. Administration: 8a, Medical Care; 8b, Maternal Care. 9. Health Education. 10. Child Hygiene; 10a, General; 10b, Dental Hygiene; 10c, Mental Hygiene; 10d, Nursing.

The book consists of abstracts of papers published during 1939 and 1940. Following a number of the abstracts are personal evaluations by Dr. Geiger. The articles abstracted are gathered rather widely from the literature. Many of them appeared in journals and foundation reports which the average worker in public health might not see. The book is thoroughly indexed by subject and author. This book should serve a very useful purpose and enable busy health workers to keep abreast of an ever increasing literature.—H. W. BROWN.

# AMERICAN SOCIETY OF PARASITOLOGISTS

SIXTEENTH ANNUAL MEETING, PHILADELPHIA, PA.

DECEMBER 30 AND 31, 1940, AND JANUARY 1, 1941

## *Minutes of Sixteenth Annual Business Meeting*

The sixteenth annual business meeting of the Society was held at the Hotel Philadelphian on December 31, 1940, following the annual Parasitologists' Luncheon which was attended by 158 members and guests. The meeting was called to order at 2:00 PM by President David H. Wenrich who expressed his gratification for the large attendance, including seven former Presidents of the Society, Henry B. Ward, W. W. Cort, William A. Riley, W. H. Taliaferro, George R. LaRue, F. C. Bishopp and Horace W. Stunkard.

### I. REPORTS OF OFFICERS

1. The report of the Secretary was presented. As of December 5th, there were 479 members on the roll, including 48 in arrears. Sixty new members were elected during 1940. The deaths of the following members occurred during the past year: J. Lee Kirby-Smith, practising physician, January, 1940, in Jacksonville, Florida. Harvey P. Barret, retired physician, July 30, 1940, in Charlotte, N. C. Ralph K. Collins, Field Director in the International Health Division of the Rockefeller Foundation, October 1, 1940, in New York City.

The secretary reported the following actions of the Council at its meeting held on December 29, 1940:

(1) Amendment of the By-Laws in regard to the allotment of time for papers on the program to read, "If the program is crowded, the maximal allotment of time may be reduced to 10 minutes."

(2) Passage of a resolution to go on record as not approving the description of new genera and new species in abstracts for the program.

(3) Passage of a new By-Law to provide for the management of the Endowment Fund as follows:

"Council shall select a Custodian of the Endowment Fund and two associates to whom it may delegate responsibility for management of the Fund. The Custodian shall make an annual accounting to Council and such other reports as Council may request. The approval of two of the three custodians shall be necessary for the purchase, sale or exchange of securities. One of the three custodians shall be the Treasurer of the Society and his signature shall be required on all vouchers of expenditure from the Fund."

(4) Proposal to the Society that the Constitution be amended to provide that the Chairman of the Editorial Committee shall be ex officio a member of the Council. Upon motion the report was approved and placed on file.

2. The report of the Treasurer was presented by Dr. Gilbert F. Otto. A favorable operating margin for the year of \$141.80 wiped off the book deficit of \$106.42 remaining from the past 5 years and also the \$32.75 current deficit on the 25-volume Index, leaving an unexpended balance of \$2.62. The Treasurer pointed out that his estimate of 1941 income was conservative, and perhaps pessimistic, being based on the assumption that the Journal would lose 55 more non-member subscribers on account of war conditions abroad. During 1940 there was a decrease of 27 in non-member subscriptions from foreign countries. According to this estimate the Society would incur a deficit of approximately \$465 in publishing a 600-page Journal during the coming year.

Upon motion the report, certified as correct by the Auditing Committee, was accepted with a vote of thanks to Dr. Otto for his services as Treasurer during the past four years.

## II. REPORTS OF COMMITTEES

1. The report of the status of the Secretarial Fund was presented by Dr. Norman R. Stoll, Custodian. Upon motion the report, certified as correct by the Auditing Committee, was accepted and placed on file.

2. The report of the Editorial Committee was presented by Dr. Norman R. Stoll, Chairman. "The JOURNAL OF PARASITOLOGY itself (Volume 26 in 6 numbers of 539 pages, and the December Supplement of 48 pages), regularly issued on schedule, constitutes the main report of the Editorial Committee for 1940. In addition a 25-Year Index to the Journal is well advanced toward final publication. The Author Index of 3,397 items is in page proof, the general Subject Index is in part in type, and the Host Index is ready for checking of copy for accuracy. The 25-Year Index will constitute, at completion, a book of about 200 pages, at a pre-publication price to members of the Society of \$1.00, and a uniform post-publication price of \$2.00. Its pre-publication sale to date promises its success financially."

Upon motion the report was accepted with a vote of thanks to the Editorial Committee.

3. The report of the Biological Abstracts Committee was presented by Dr. George L. Graham, Chairman. It was pointed out that the loss of foreign subscribers to Biological Abstracts on account of the spreading war abroad makes it imperative, now more than ever before, that American biologists support Biological Abstracts if it is to maintain its standard of publication. Upon motion the report was accepted.

4. A condensed report of the Committee on Nomenclature was given by Dr. Paul D. Harwood, Chairman. It was the opinion of the Committee that under the International Rules of Zoological Nomenclature *Trichuris* rather than *Trichocephalus* is the valid generic name, and that *Diectophyma renale* is the valid name for the giant kidney worm. (The complete report of the committee is appended.)

Upon motion the report was accepted.

## III. REPORTS OF REPRESENTATIVES OF THE SOCIETY

1. Representatives on the Council of the American Association for the Advancement of Science. Since the proceedings of the Council will be published in *Science*, Dr. LaRue offered no report at this time.

2. A brief report of the representatives on the Council of the Union of American Biological Societies was presented by Dr. Earl C. O'Roke. The financial straits of Biological Abstracts are not as serious as was feared several months ago and it is expected that regular publication can be maintained without any curtailment during the coming year. Forty-three lapsed foreign subscriptions are being continued through underwriting by philanthropic agencies in this country.

Upon motion the report was accepted.

## IV. NEW BUSINESS

The following two amendments to the Constitution proposed at the fifteenth annual meeting in Columbus were adopted by unanimous vote:

JOURNAL OF PARASITOLOGY. This JOURNAL, the property of the Society, is its official organ. Responsibility for its conduct shall rest with the Council which shall select the editorial staff and set the price of subscription.

ENDOWMENT FUND. Provision is made for the establishment of a permanent Endowment Fund, the principal of which may be expended only by a three-fourths vote of all members of the Council and approval by a three-fourths vote of the members of the Society present at a regular



meeting. The Council shall be entrusted with the maintenance of the fund, and the use of the income therefrom.

Upon a motion in accordance with the recommendation of the Council, it was voted that the selection of the 1941 meeting place be held in abeyance and that the Council be entrusted to decide the meeting place.

The Secretary presented the Council nominations for officers of the Society for 1941, as follows: President, James E. Ackert; Vice-president, Justin Andrews; Treasurer, for two years, Lloyd E. Rozeboom; Members of the Council, for four years (to succeed Justin Andrews and Norman R. Stoll), Donald L. Augustine and Norman R. Stoll; Members of the Editorial Board, for four years (to succeed H. E. Ewing, J. F. Kessel and H. J. VanCleave), E. Harold Hinman, Richard P. Hall and Justus F. Mueller; Representatives on the Council of the American Association for the Advancement of Science, George R. LaRue and William A. Riley; Representatives on the Council of the Union of American Biological Societies, George L. Graham and Earl C. O'Roke. There were no further nominations and upon motion, duly seconded, the Secretary was instructed to cast one ballot for the nominations as presented.

A vote of thanks was extended to Dr. Robert M. Stabler, local representative of the Society in Philadelphia, for his services in making arrangements for the luncheon and the demonstration program.

There being no further business, it was voted to adjourn at 3:00 PM.

Respectfully submitted,

O. R. McCoy, *Secretary*

#### CHANGES IN MEMBERSHIP OF THE AMERICAN SOCIETY OF PARASITOLOGISTS SINCE PUBLICATION OF THE MEMBERSHIP ROSTER, DECEMBER SUPPLEMENT, 1939

##### *New Members Elected*

Josephina Acosta	Lyman P. Frick	Francis W. Ludwig
Cyrus V. Anderson	Deane P. Furman	Everett E. Lund
John P. Barrett	Tomas M. Gan	Walter S. Lundahl
José G. Basnuevo	Chauncey G. Goodchild	José F. Maldonado
Jonas L. Bassen	Seymour Hadwen	Rustum Maluf
Enrique Beltran	Merle F. Hansen	Manuel Martinez-Baez
Marjorie Biddle	Aaron B. Hardcastle	Joseph C. McCaffrey
Virginius E. Brown	William B. Hopp	Charles W. McNeil
Martha Bunting	Herbert S. Hurlbut	Banner Bill Morgan
Harold Burnstein	Kathleen L. Hussey	Donald V. Moore
Arthur A. Case	Nobutaro Ishii	Emma S. Moss
S. L. Chang	Harry A. Jankiewicz	Ross F. Nigrelli
Frederick Coulston	Frances Jones	José Oliver-Gonzalez
Helen Churchill	Kenneth C. Kates	Eddy A. Palmer
Joseph S. D'Antoni	Walter Kessler	James P. Parker
Zacarias de Jesus	Fred J. Kohlruss	Eduardo A. Pequeño
Elaine T. Delaune	Emil Kotcher	Trinidad P. Pesigan
William E. De Turk	Robert E. Kunz	Collins A. Pipkin
S. Allen Edgar	Raymond L. Laird	Karl R. Pomrenke
Leslie L. Eisenbrandt	William K. Lawlor	David Price
Kary C. Emerson	Herman Lent	Robert J. Reiber
Albert M. Fallis	Alice Smith Leonard	W. Malcolm Reid
Marion M. Farr	Arthur Levin	Leslie W. Remley
Anna M. Fisher	Ralph D. Lowell	Robert C. Rhodes



Kenneth I. Rhude  
 Anthony T. Rozycki  
 Robert L. Rutherford  
 John G. Salsbury  
 Aaron Seamster  
 Herman A. Shelanski  
 Victor Sprague

Glenwood M. Spurlock  
 Harry H. Stage  
 Dorothy E. Strahan  
 Vivian Sweibel  
 John H. Tetley  
 John E. Tobie  
 Charles M. Vaughn

Harold E. Wallace  
 Helen J. Werby  
 Evaline West  
 Francis M. White  
 Raymond W. Wilhelmi  
 Ruby A. Wortham  
 Herman Zaiman

### Resignations

Veronica Armaghan  
 Alice T. Ashton  
 Charles C. Bass  
 John A. Cameron  
 Walter C. Earle

Charles S. Gibbs  
 George D. Jelen  
 Francis B. Johnson  
 E. Elizabeth Jones  
 Miriam Scott Lucas

Helen Stewart Lyford  
 Rafael Rodriguez-Molina  
 Lauren E. Rosenberg  
 Fred L. Soper  
 J. Paul Visscher

### Deaths

Harvey P. Barret  
 Ralph K. Collins

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## SUPPLEMENT TO THE REPORT OF THE SIXTEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

### REPORT OF THE COMMITTEE ON NOMENCLATURE

#### *Trichuris* Roederer, 1761 vs. *Trichocephalus* Schrank, 1788

Since the name *Trichuris* Roederer, 1761, clearly antedates all other technical names proposed since 1758 for the genus of the whipworms, this name should be employed, providing the requirements laid down in the International Rules of Zoological Nomenclature are met by the original description. These requirements are few.

The name was published and accompanied by a description of the parasite (Art. 25a) as two brief quotations from the original demonstrate (Roederer, J. G. [in Secretary's abstract by Wagler.] Götting. Anz. gelehrt. Sachen (1761-62) 25 St. 1: 243): "Bey eben dieser Zusammenkunft las der Hr. Leibmed. und jetziger Prorektor Röderer eine Abhandlung vor, welche eine gewisse bisher noch nicht beschriebene Art Würmer im menschlichen Körper betrifft. Sie ist den dreyen bisher bekannten, dem runden Wurme, dem Bandwurme, dem Spulwurme, beyzufügen, und wird, ihrer Gestalt gemäss von Hrn. R. *Trichuris* (Haarschwanz) genannt;" and (p. 245): "Diese neue Art von Würmern der Eingeweide liesse sich also nach der Linnäischen Art so beschreiben: Corpus teres, longa proboscis filiformis, genitale curuarum, emineus, rectorum, apertura lateralis." Since Roederer (1761) stated he was describing the parasite "after the Linnæan manner" i.e., the binary manner (Stejneger, 1924. Smithsonian Misc. Coll. 77 (4): 1-21), it may be assumed that he "applied the principles of binary nomenclature" as required under Art. 25b (International Rules of Zoological Nomenclature).

Some authors have rejected the name *Trichuris* because it was not used in a binomial combination, but Article 2 of the Rules states: "The scientific designation of the animal is uninominal for subgenera and higher groups," and in Opinions 20, 24, and particularly 46 the International Commission on Zoological Nomenclature has consistently recognized uninominal generic names employed in a binary sense regardless of whether or not the name was used in a binomial combination. Therefore, the name in question should not be rejected because it is uninominal.

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At least one author has claimed that the name *Trichuris* Roederer, 1761 was used as a specific name. However, the evidence contained in the original description indicates that Roederer was proposing a generic name. *Trichuris* is a noun, not an adjective such as Linnaeus usually used for specific names, and furthermore, Roederer's description "nach der Linnäischen Art" is precisely in the style which Linnaeus (1758, p. 648. "Ascaris. *Corpus* teres, filiforme, continuum, utraque extremitate attenuatum.") employed when he wrote the diagnosis of the genus *Ascaris*, and it does not agree with the style employed for specific diagnosis. Authors contemporary with Roederer (Goeze: 1782, Versuch eine Naturgeschichte der Eingeweidewürmer thierischer Körper, Blankenburg) as well as other writers treated *Trichuris* as a generic name and renamed the genus, *Trichocephalos* (= *Trichocephalus*) hair-headed, because of the anatomy of the parasite. If some doubt whether or not Roederer intended to use the name *Trichuris* in a generic sense, in the abstract of his original paper, a later contribution by the same author should remove this doubt. (Roederer, J. G. and Wagler, C. G. 1783, Tractatus de morbo mucosa. Denuo recusus annexaque praefatione de *Trichuridibus* novo vermium genere, editus ab Henrico Augusto Wrisberg. Gottingae). The above title proves unquestionably that *Trichuris* was used as a generic name by the original author.

Accordingly the name *Trichuris* Roederer, 1761 appears to be available under the International Rules, and in our opinion it should be used as the correct name for the whipworms. *Trichocephalos* Goeze, 1782 and *Trichocephalus* of authors are synonyms.

#### COMMITTEE ON NOMENCLATURE,

PAUL D. HARWOOD, *Chairman*

B. G. CHITWOOD

ALLEN MCINTOSH

#### The status of *Diectophyma*, *Diectophyme*, and *Eustrongylus*

Collet-Meygret (1802, J. Phys. Chimie, Hist. Nat. et Arts, 55: 458-464) proposed the name, *Diectophyme*, for a worm from the kidney of a dog. He gave an extended description of the worm and an illustration of a dissection. When he first presented this paper before the "Société philomatique de Paris" he stated that he felt sure it was a new genus, but he would leave the point to a study by specialists of this order of animals. In a section of this paper labelled "Remarque" (p. 463) the author states that the society appointed two members to verify his observations and determine whether it was essentially different from other known worms. The opinion arrived at is set forth in the original article as follows:

"Ils ont en conséquence jugé, comme moi, que cet individu devoit former un genre nouveau, très-voisin de celui des ascarides. J'établis donc ce nouveau genre, auquel je vais essayer de donner une dénomination, et d'assigner des caractères.

"Quant à la dénomination, comme elle doit être tirée des attributs qui, en même temps qu'ils offrent quelque chose de constant, frappent d'abord nos sens, j'ai préféré la faire dériver du nombre des tubercules; et comme il y en a huit à la tête et huit à la queue, sans égard pour leur disposition, j'ai adopté le mot *diectophyme* (author's italics) composé de *di*, venant de *dis* (deux fois), *octo* (huit), *phyma* (tubercle), expression qui, je crois, remplit le but que je me propose.

"Voici quels sont les caractères du genre *diectophyme*:

"*Corps* allongé, cylindrique, articulé, à extrémités mousses, garnies chacune de huit tubercules; bouche terminale, anus également terminal; réunion des deux sexes (1)."

It is obvious from the description appearing in the original article that the worm which Collet-Meygret discussed was the form which has been known variously as *Diectophyme renale*, *Diectophyma renale*, and *Eustrongylus visceralis*. Furthermore, it is obvious that Collet-Meygret intended to employ the term "*diectophyme*" in a generic sense as he employs the phrase "*du genre diectophyme*" in the quotation cited above. Therefore, it is the opinion of your committee that the conditions laid down under Article 25 (the law of priority) of the International Rules of Zoological

Nomenclature were fully met by Collet-Meygret, and the name "*diotophyme*" should date from his paper. This opinion has no bearing on question of the emendation of the original spelling.

Some authors (Leiper, 1926) have rejected "*diotophyme*" on the grounds that the original author was employing it as a vernacular term instead of a scientific name. Although the orthography suggests that Collet-Meygret intended to use "*diotophyme*" in the vernacular, the statements which are quoted above, appearing in the original description indicate that the author was proposing a technical name in conformity with the principles of binary nomenclature. The error appearing in the original orthography may be corrected under the authority of Article 19 of the International Rules of Zoological Nomenclature. Acting in accordance with this authority, your committee recommends the adoption of the generic name *Diotophyma* Collet-Meygret, 1802, as emended by Lamouroux in 1824. *Eustrongylus* Diesing, 1851, becomes a synonym under the law of priority.

Opinions 26, 27, 34, 41, 61, and 63 indicate that the policy of the commission has been to recognize emended spellings in those cases where the derivation of the word is clearly indicated, but as Stiles and Baker (1935, National Inst. Health, U. S. Pub. Health Serv., Bull. No. 163: 981) have stated, there exists a difference of opinion among zoologists "as to how far the principle of emendation should be applied." Consequently, to obtain uniform usage your committee recommends that the American Society of Parasitologists request the International Commission on Zoological Nomenclature to place *Diotophyma* on the Official List of generic names.

Regarding the specific name of the kidney worm of carnivores, the following may be considered, namely: *Renales* Goeze, 1782; "*Gigas*," a group name employed by Goeze, 1782; *visceralis* Gmelin, 1790; and *renalis* Gmelin, 1790. The specific name *gigas* Rudolphi, 1802, which has been used by authors, is clearly antedated by the names listed above.

The passage by Goeze (1782, p. 39) involving the names in question is quoted below.

"I. Geschlecht (Genus)  
der Rundwurm (Ascaris).

1. Der Riese: *Gigas*. Dieser begreift die grössern Arten unter sich.
  - a) der Pferde: die grösste Art, die ich kenne.
  - b) der Menschen: die eigentlich sogenannten Spulwürmer: *Ascaris Lumbric.* Linn.)
  - c) der Schweine: schwächlicher und elastischer: mit einer, längs dem Rücken laufenden Orangegelben Streife.
  - d) der Kälber: noch länger, und dünner, als die vorigen.
  - e) Nierenrundwürmer, *Renales*.
  - f) der Seehunde: *Phoca*.
2. Der Mittelrundwurm: *Teres*) von Mittelgrosse, wie Darmsayten. . . .
3. Der Madenrundwurm: *Asc. minutior*. . . ."

Later in the same article (p. 62) Goeze states, "Nach meinen Erfahrungen hab' ich drey Hauptklassen dieses Wurms kennen lernen: die grosse: die mittlere; und die kleine. Die erste nenne ich den Riesen, *Gigas*; die zwote den mittelrundwurm, *Teres*; die dritte den Madenrundwurm, *minutior*." Because of the above statement we believe that the names *Gigas*, *Teres*, and *minutior* are group names which do not have nomenclatural status. In this respect they are similar to the group names. *Didelphys*, *Tridelphys*, *Tetradelphys*, and *Polydelphys* which modern authors use to subdivide the genus *Physaloptera*. (See Ortlepp, 1937, Onderstepoort J. Vet. Sc. and Animal Industry, 9: 71) and which possess no nomenclatural status. The case is also similar to the generic subdivisions employed by Linnaeus in the tenth edition of the *Systema Naturae* (pp. 425-433). Linnaeus divided the genus *Gryllus* into sections, namely, "*Mantis*," "*Acridia*," "*Bulla*," "*Acheta*," "*Tettigonia*," and "*Locustia*." In Opinion 124, the International Commission on Zoological Nomenclature has ruled: "The various subdivisions of genera published by Linnaeus in 1758 are

not to be accepted as of this date (1758) as of subgeneric value under the International Rules." In view of these examples it seems justifiable to ignore Goeze's group names.

Although it may be argued that Goeze did not make the combination *Ascaris renalis*, the table, which is quoted above, indicates that his usage was both binary and binominal. Furthermore, "*Renales*" was printed in the same type as the specific name, "*Lumbric*. Linn." in Goeze's book, a circumstance which indicates that it was used as a technical name.

A comparison of Linnaeus (1758) with Goeze (1782) demonstrates that there is no essential difference between the two styles. The fact that Goeze was somewhat less consistent than Linnaeus is unfortunate but not important from the standpoint of nomenclature. Interpreting Goeze's style in view of the historical situation, we must conclude that it was binominal in the Linnean sense. Therefore, we consider that the specific name "*Renales*," Goeze, 1782, is available under the International Rules of Zoological Nomenclature, and since it is the oldest available name, the correct name of the kidney worm of carnivores is considered to be *Diectophyma renale* (Goeze, 1782).

If we were to assume that the word "Gigas" as employed by Goeze is available as a specific name under the rules, this name must be applied to all the various forms of nematodes which that author listed under the heading, "Gigas," from (a) to (f). The name "*Renales*" in that case is of lower rank, and therefore, it is employed as a subspecific name and it becomes available under the rules, since, according to Article 11, subspecific and specific names are of the same value.

The specific name *visceralis* which is the next available name, was proposed by Gmelin (1790). On the following page of the same work Gmelin also used the name *renalis* for the same parasite, namely, the kidney worm of carnivores. Therefore, *visceralis* Gmelin, 1790, is a renaming of *Diectophyma renale* and becomes a synonym.

COMMITTEE ON NOMENCLATURE,  
PAUL D. HARWOOD, *Chairman*  
B. G. CHITWOOD  
ALLEN MCINTOSH

## AMERICAN SOCIETY OF PARASITOLOGISTS

### ANNOUNCEMENT OF PLACE OF SEVENTEENTH ANNUAL MEETING

Acting under authority voted at the sixteenth annual business meeting in Philadelphia, the Council has decided that the seventeenth annual meeting of the American Society of Parasitologists shall be held in Dallas, Texas, in conjunction with the meeting of the American Association for the Advancement of Science. Sessions for the presentation of papers will be scheduled for Monday, Tuesday, and Wednesday, December 29, 30 and 31, 1941.

O. R. McCoy, *Secretary*



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## THE EFFECTS OF FOUR SPECIES OF LARVAL TREMATODES UPON THE LIVER AND OVOTESTIS OF THE SNAIL, *STAGNICOLA EMARGINATA ANGULATA* (SOWERBY)

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A snail may shed large numbers of cercariae when confined in the laboratory for study, and yet show no signs of ill-health as a result of the presence of large numbers of parasites within its body. Brackett\* (1940) stated that lack of nourishment on the part of the snail decreased the production of cercariae. When the snail was fed, it again shed cercariae.

Faust (1917) devoted some attention to the effect of larval trematodes upon several species of snails. Cytological evidence was presented to show that liver cell changes took place following invasion of the snail by the parasites. He stated, "In an examination of living material and sections of infected mollusk liver tissue, no infection was found to be so light that the host was unharmed."

Wesenberg-Lund (1934) described the effect of larval trematodes upon four species of snails. He recorded one "tumor-like" growth in a snail which he attributed to the effect of the larval parasites and stated that "The liver was almost destroyed."

Rothschild (1938) concluded that snails of the genus and species *Peringia ulvae* when parasitized by trematodes were smaller than other individuals of the same age that were not parasitized.

The present study was limited to the effect of four species of larval trematodes upon the liver and ovotestis of *Stagnicola emarginata angulata* in an effort to determine: (1) whether different species of parasites had appreciably different effects upon the same species of snail host; (2) what effect the presence of large numbers of parasites had upon the ovotestis and the tubules of the liver; and (3) whether or not parasites were found within the lumina of the liver tubules or ovotestis.

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\* Acknowledgment is made to Dr. Sterling Brackett, University of Wisconsin, who provided the snails used in this study and identified the species of trematode parasitizing each snail.



## MATERIALS AND METHODS

All the snails used in this work were *Stagnicola emarginata angulata* and were collected from Douglas Lake, Michigan. The cercariae from each snail were identified while alive and the snail was then extracted from the shell. The upper half of the snail, constituting liver to the unaided eye, was detached from the rest of the animal and the two pieces fixed together in Gilson's fluid. The specimen infected with *Plagiorchis muris* was fixed in Goldschmidt's fixative.

After fixation the specimens were transferred to dioxan until the time when they could be embedded in paraffin. The paraffin blocks were sectioned 8 to 14 microns thick, affixed to slides and stained in Semicon's carmine. When a strong, fresh stain was used, one hour in the stain was adequate to produce a sharp differentiation of the tissues. After staining, the slides were washed in 70 per cent alcohol, dehydrated in dioxan and mounted in damar.

The cercariae coming from the infected snails were identified respectively as: *Cercaria laruei* (Cort and Brooks, 1928), *Plagiorchis muris* (McMullen, 1937), *Cercaria yogenae* (Cort and Brackett, 1937) and *Diplostomum flexicaudum* (Cort and Brackett, 1937). All snails studied had been shedding only one kind of cercariae at the time that they were killed. A snail which was not shedding cercariae into the water at the time of examination and in which no cercariae were found after sectioning was studied for comparisons with the infected snails. Careful examination of the entire liver of each snail was made.

## DATA

The liver of an uninfected snail identified as *Stagnicola emarginata angulata* was made up of a fairly compact mass of tubules held in place by a loose network of connective tissue, the latter of which was not completely continuous. The whole mass of the liver was confined by a distinct and continuous limiting membrane. Toward the anterior end of the liver larger thin-walled ducts with a villi-like mucosa and rather large lumina connected the liver to the intestine. These have been designated liver ducts. The tubules of the liver itself were two cells thick with a distinct lumen in each tubule. An amorphous material that stained lightly with Semicon's carmine often was found in the lumen of the liver tubules. Light staining areas in the cells making up the inner wall of the tubules indicated that some soluble substance had been present which the dioxan or alcohol had removed.

In the uninfected snails the liver tubules were distributed evenly within the liver. In a cross-section in the upper third of the liver there were found to be 112 disconnected liver tubules. This number was not an average; it was recorded because it was the count on a section similar to ones studied in infected snails later in this paper.

The ovotestis of the uninfected snail was embedded within the body of the liver and was made up of a very thin-walled sac which extended a considerable distance within the liver. Within the ovotestis numerous partially developed eggs were to be seen attached to the wall. There were no sperm present, but that was explained by the fact that the snails were killed in August after the male phase had been completed and the animals were functional females.

A snail infected with *Cercaria yogena* was prepared for detailed study. One parasite was found in the small upper end of the liver and three more somewhat farther along lying just under the limiting membrane. In neither of these sites were the parasites in close contact with the liver tubules. Farther forward six specimens were found lying loosely about the hermaphroditic duct in the connective tissue which was rather abundant in that area.

At the place where the intestine reached its most posterior extent and bent upon itself toward the anterior, there were nine parasites lying alongside the intestine. Slightly anterior to this point the hermaphroditic duct came to lie between the two loops of the intestine, and the three tubes were loosely held together by connective tissue. From this point forward the parasites became more numerous and were located in this connective tissue. At the smaller anterior or lower end of the liver a count was made of liver tubules and parasites in cross-section. The section selected contained 29 liver tubules and 58 parasites.

This snail had a medium to light infection, and the parasites remained in the central area of connective tissue previously mentioned. None was found far enough toward the periphery of the liver to be surrounded completely by liver tubules with the possible exception of the two groups on the extreme upper end of the liver. The parasites did not lie against the liver tubules and none was within the lumen of either a liver tubule or a reproductive organ.

Only ova were present within the ovotestis, but the snail had been killed in August, as had the previous one. Neither the liver nor the ovotestis showed obvious signs of injury in this snail.

A snail infected with *Cercaria lariuei* was carefully examined for the characteristics of that infection. The snail chosen was heavily infected so that an estimate of the number of parasites present was not attempted. At the tip of the liver beyond the ovotestis where no tubes or open spaces provided a natural avenue for penetration, four parasites were present among the tubules. A short distance toward the anterior the parasites became numerous and were concentrated in the central area of the liver, while the liver tubules were found to occupy the periphery of that organ.

Thin-walled sacs were present in the center of the liver and could be identified by location and nature of the sac wall as ovotestis, but neither

eggs nor sperm were present. A few cells were present in the sac. Similar cells had been identified as primordial germ cells in other material. The snail appeared to be sterile. No parasites were found within the lumina of the ovotestis, the various ducts or liver tubules.

A complete cross-section contained 76 parasites and 49 liver tubules. The parasites were rather small and the liver tubules were smaller than in either the negative or in the snail infected with *Cercaria yogena*. The network of connective tissue of the snail was evident only in the peripheral areas of the liver between the tubules where there were no parasites. It seemed probable that the presence of the parasites and their migrations had destroyed the central area of connective tissue which was present in the uninfected snail liver.

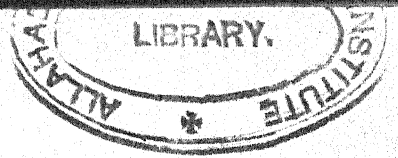
The snail that was infected with *Plagiorchis muris* larvae was heavily parasitized. Parasites were found among the liver tubules even far up at the tip of the liver. The tendency observed in the infection by *Cercaria yogena*, for the sporocysts and rediae to stay in the open spaces or along the outside of the loops of the intestine was not discernible in this infection. The large number of parasites present might have influenced the more general distribution among the liver tubules. The parasites did not crowd against the liver tubules, however, but shared the available space within the liver in a fashion similar to the distribution pattern of the liver tubules. The site of heaviest infection was an area that adjoined the intestine. This suggested that the path of ingress had been along the intestine.

Careful search was made for the ovotestis and related ducts, but no unmistakable part of that gland was found. The parasites were very numerous in that part of the liver where the ovotestis was imbedded in other specimens. This snail was killed and fixed at the same time of year as the other specimens in which an ovotestis was conspicuous.

A survey of the entire cross-section in the middle of the liver was made. There were 149 liver tubules and 117 parasites present. In one area where a liver tubule was flanked closely on either side by a parasite, the one end of the tubule was open, and the external cell layer of the liver tubule was absent. This was an exception and was the only one seen in thousands of liver tubules studied.

The parasites in this snail were of various sizes with numerous large cyst forms. The invasion among the liver tubules was more pronounced than in the other snails studied and the absence of an ovotestis was remarkable.

The infection with *Diplostomum flexicaudum* was heavy. An entire cross-section contained 48 liver tubules and 101 distinct parasites in addition to material not countable. The last was probably parts of cercariae already freed from their mother cyst. The parasites were crowded in amongst the liver tubules, but were thickest in the central area of



the liver. Many of the liver tubules were normal in appearance, but those in the central area of the organ where the parasites were thickest were smaller than the rest and took the stain more deeply than did the larger tubules at the periphery. Where parasites actually touched liver tubules in this snail, the outer layer of cells of the tubule did not appear modified by the proximity of the cyst.

The ovotestis was identified in this snail, but no eggs or sperm were present. The thin-walled sac was not invaded by flukes and the lumen was of normal size. Although the organ contained no eggs, there were present peculiar brown structures of irregular shape and a few cells that appeared to be primordia of germ cells.

#### DISCUSSION

The avenues of invasion of the snail by the larval trematodes, as far as could be determined, followed up the intestine and hermaphroditic duct and from thence into the body of the liver. The majority of the parasites remained in the central area of the liver. This was observed in the infections produced by four different species of trematodes. The parasites were found in the loose connective tissue between liver tubules and surrounding the intestine, ovotestis and hermaphroditic duct, but not within any of these ducts.

Variations in the different infections might well have been the result of differences in severity of infection. The *Cercaria yogena* infection did not seem to have harmed the snail as greatly as did the other three, but the number of parasites in the snail in this infection was markedly fewer. This infection was studied at greater length than the others because it was light enough to suggest which areas in the snail were first sought by invading trematodes.

The effect on the liver tubules was hard to demonstrate. A glance at the liver of a heavily infected snail gave the impression that the parasites had taken over the central area of the liver, and that the number of liver tubules was fewer as compared with an uninfected snail. These impressions were not quantitatively verified. The liver tubules in the center of the liver in heavily infected specimens were smaller than those on the same section at the periphery. In one case (*Plagiorchis muris*) there was no other obvious reason for the breakdown of the liver tubule in the center of a section than the close proximity on both sides of large parasites. The difference in staining of the tubules that were surrounded by parasites was probably an indication of changes or even degeneration of the liver tubule.

The ovotestis in both the uninfected snail and in the one lightly infected with *Cercaria yogena* appeared capable of producing ova. There was no evidence of any partially developed eggs in the snails infected with *Cercaria laruei* and *Diplostomum flexicaudum*, although the thin-walled



ovotestis was present and intact. The snail harboring the infection of *Plagiorchis muris* was unique in that there was no trace of the ovotestis. It seemed probable that heavy infections of trematode larvae had rendered the latter three snails sterile.

In none of the snails studied was there any evidence that the parasites penetrated into the lumen of any of the various ducts present.

#### SUMMARY

1. Serial sections were made of the livers of five specimens of the freshwater snail *Stagnicola emarginata angulata* (Sowerby).

2. One snail was not shedding cercariae at the time that it was killed, and microscopic examination of the whole animal showed that no parasites were present.

3. One snail was infected rather lightly with *Cercaria yohana*.

4. Three snails were heavily parasitized by *Cercaria laruei*, *Plagiorchis muris* and *Diplostomum flexicaudum* respectively.

5. No appreciable difference in method or effect of invasion by the four species of trematodes in the same species of snail could be distinguished.

6. The effects of a heavy infection was to reduce the apparent number of liver tubules and to limit the liver tubules to the periphery of the liver. The parasites filled the central area. In only one case did there seem to be evidence of destruction of liver tissue by the proximity of parasites.

7. In the snail infected with *Cercaria yohana* the ovotestis was apparently normal as compared with that of the uninfected snail. The ovotestis was present but contained no developing eggs in the two snails infected with *Cercaria laruei* and *Diplostomum flexicaudum*. The ovotestis was not present in the snail infected with *Plagiorchis muris*.

8. In no instance was a parasite found within the lumen of liver tubule, ovotestis, hermaphroditic duct, or intestine.

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THE REDISCOVERY TOGETHER WITH THE MORPHOLOGY  
OF THE LEECH, *BRANCHELLION RAVENELII*  
(GIRARD, 1850)

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Through the courtesy of Dr. Ross F. Nigrelli, of the New York Aquarium, I have been able to examine sixty-six specimens of *Branchellion ravenelii* (Girard, 1850), collected March 3, 1939, at Lemon Bay, Florida. Thirty-two of these specimens were taken from the ventral external surface of *Dasyatis hastatus* (DeKay, 1842),<sup>1</sup> the remaining twenty-four, from the same location on *Amphotistius sabinus* (LeSueur, 1824).

Dr. S. C. Ball, Curator, Peabody Museum of Natural History of Yale University, kindly sent me for my examination all of Verrill's leech collection which the former was able to assemble.

*Branchellion ravenelii* is a leech, PISCICOLIDAE, first described by Girard, 1850, from a skate taken at Charleston, South Carolina. Verrill (1874: 624) reported the species from "Vineyard Sound, on a stingray (*Myliobatis freminvillei*) [= *Aetobatus freminvillei*], in several instances, a number usually occurred together."

In describing the species Girard mistook the anterior for the posterior end and was unaware that the animal under consideration was a leech. His nebulous description, in part, follows:

In whatever light we may view the animal kingdom, whether forming a series, one uninterrupted, from simple to more complicated forms, or composed of groups independent from each other, it remains as a fact, that the animal now under consideration, combines in itself, the general character of two distinct groups. Its general form is framed upon the plan of *Piscicola*, but, in addition, it has lateral appendages, (gills) reminding us of the same organs among annelids proper.

The body is flat, elongated, terminated at both extremities by a disk, on which the animal crawls about, in the fashion of the earth measurer, caterpillar, and leeches.

Indeed the body seems more alike that of a leech, thus showing a third group to which it bears a great analogy.

At the conclusion of this paper, Professor Agassiz stated, that it is very rare to find intermediate animals existing between types. Now this specimen exhibits characters of three groups, and thus plainly shows that these characters can not have much value as the bases of types.

Although Diesing (1859: 482-483) listed the species (*Branchiobdella ravenelii*) in "Systema Helminthum," he did nothing more than copy Girard's confused description. Pratt included the species in his "Manual of the Common Invertebrate Animals." The foregoing are the only

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<sup>1</sup> The fish nomenclature here followed is that adopted by Jordan et al, 1930.

records of *B. ravenelii*. No further data regarding the species are available except in the form of the references of Diesing, Verrill, and Pratt, none of which deal with internal morphology.

Upon an examination, however, of Verrill's material, in which there was one specimen clearly belonging to the genus *Branchellion* and labeled "*Phyllobranchus ravenelii*, Vineyard Sd.," it appears questionable that he collected *B. ravenelii*. Instead, Verrill's specimen should have been assigned to the well established type species, *Branchellion torpedinis* Savigny, 1820, since it possesses thirty-three pairs of lateral, broad, foliaceous, lobed or scalloped, branchiae. If the figure accompanying Verrill's article (Pl. XVIII, Fig. 89<sup>2</sup>) is based on the specimen examined, he erred, because in the drawing only thirty-two pairs of such branchiae are shown. Because only one specimen was available, it was not possible to dissect or section it in order that a comparison between the reproductive systems of *B. ravenelii* and *B. torpedinis*, as worked out by Sukatschoff, could be made.

The most striking characteristic of *B. ravenelii* consists in its having thirty-one pairs of lamellar branchiae along each side of the posterior  $\frac{3}{4}$  of the body. It is also to be noted that the posterior sucker (anterior of Girard and of Diesing) possesses about 400 cupules situated on its ventral surface. The body is sharply demarcated into trachelosomal, pre-urosomal, and urosomal regions and the sucker belonging with the first and third. The two anterior rings of the urosome extend forward over the smaller anterior trachelosome, forming the pre-urosomal or "collar" of some authors.

For study the material was prepared in the same manner as described in a previous publication by the writer (1940: 335). The species under consideration consequently corresponds to the synonyms and the following diagnosis:

(Figs. 1-3)

Synonyms: *Branchellion revenelii* (Girard, 1850); *Phyllobranchus ravenelii* Girard, 1850; *Branchiobdella ravenelii* Diesing, 1859; *Branchellion reveneli* Pratt, 1916.

Body is sharply divided into two regions; the trachelosomal comprises about  $\frac{1}{4}$  the total length and is composed of twelve segments, the urosomal consists of twenty-two somites and comprises the remaining  $\frac{3}{4}$  the total length. Ratio of over-all length to width about 10:1. Somites in the region of the testes composed of three distinct annuli. The anterior sucker is small as compared with the posterior, very concave, cup shaped, bearing a small, exterior non-tubercular, inclined lip. Sucker eccentrically attached, in diameter equal to the transverse width of the urosome (not including branchiae). One pair of pigmented, irregular and asymmetrical eyes placed mid-longitudinally on the anterior sucker. The strongly ventrally directed posterior sucker is centrally attached to the well-defined urosomal peduncle. Sucker is circular, very concave, with a contracted rim in some specimens, in others it is discoidal. On ventral surface are about 400 cupules. Diameter of sucker equal to transverse width of urosome+lateral branchiae. Beginning with XIIIa3 and ending with

<sup>2</sup> Drawn by J. H. Emerton.

XXIV, each ring bears, on its anterior margin, a pair of lateral, broad, foliaceous branchiae; a total of thirty-one pairs. Eleven pairs of diaphanous respiratory vesicles arranged segmentally, beginning with XIIIa2 and continuing posteriorly. Distinct prepuce is present. Five pairs of testes and a seminal vesicle. Stomach possesses paired lateral expansions between adjacent testes. Male gonopore on XIa2, female on XIIa2, separated by two annuli. Copulatory area, cocoon glands and ducts conspicuous. Intestine begins a short distance posterior to the last pair of testes and is characterized by four paired lateral, posteriorly directed expansions. Rectum large. Intestinal cecum coheres at five places. Anus opens posterior to XXVII.

*Branchellion ravenelii* varies in size<sup>3</sup> within fairly wide limits. The average length is about 15.0 mm (suckers included), while the average breadth is about 5.0 mm (branchiae included) or 2.5 mm (not including branchiae) at the widest place on the body. These measurements are not standard, however, since larger and smaller sexually mature specimens were observed.

#### EXTERNAL MORPHOLOGY

The description which follows is based on the dorsal aspect; body elongated, depressed, slightly convex dorsally, entirely flat ventrally. This cross-section picture is, however, more characteristic of the urosome than the trachelosome, which is more elliptical or cylindrical. The body is sharply divided into two distinct regions (Fig. 3); the first comprises about  $\frac{1}{4}$  of the total length and is composed of twelve somites (twenty-rings), and the second comprises twenty-two somites (thirty-nine rings), representing the remaining  $\frac{3}{4}$  of the total length. Of the twelve somites contained in the trachelosome, I–IV are absorbed by the anterior sucker, and from XXVIII–XXXIV of the urosome are absorbed by the posterior sucker, leaving twenty-three somites free in the body. The dorsal surface is void of neural annulus, metameric sensillae such as are found in *Branchellion torpedinis*.

Regarding the color of *B. ravenelii*, I can do nothing more than describe the condition of specimens preserved in alcohol. The ground color varies from whitish to gray, with gray perhaps predominating. If more color and/or color markings were present in life they have been removed by the preservative.

*Trachelosomal Region.* The trachelosomal region (Fig. 2) may be conveniently divided into the anterior sucker, pre-clitellum, and the clitellum. The sucker plus one small somite represents somites I–IV. The oral sucker is small as compared with the posterior, very concave, cup

<sup>3</sup> Fundamentally, size is of little value taxonomically. But it cannot be denied that the relationship of body width to length, the body proper length (exclusive of suckers), the features separating the trachelosome from the urosome, the over-all length (body proper + suckers), the separation and the absolute and relative development of the two suckers—all of these are deserving of consideration. In other words, these characteristics seem to be of greatest value when other related forms are being compared in detail and not indicated by linear dimensions only.

shaped, bearing a small, exterior non-tubercular, inclined lip. The sucker, which in diameter is equal to the transverse width of the urosome (exclusive of the branchiae), surrounds the mouth. A single pair of pigmented, irregular and asymmetrical eyes are placed mid-longitudinally on the sucker. Somite V is composed of three small annuli; somite VI, of three annuli slightly larger in both dimensions than those of V; somite VII, of a small anterior and a larger posterior annulus; somite VIII, of two large annuli, each being equal in length to the posterior annulus of VII; somite IX, of two incomplete annuli, the anterior being about twice the length of the posterior; somite X, of two complete annuli of which the posterior annulus is twice the length of the anterior and shows a faint constriction which probably represents the tendency to divide to form a primary somite of three annuli. The clitellar region is composed of somites XI and XII, each of which is composed of three approximately equal annuli. In somite XI, beginning with a1, a2, and a3 each shows a diminution in transverse width. In XII, annuli a1, a2, and a3 are approximately equal when measured cross-wise, but a1 is nearly equal to a2 + a3 when considered longitudinally. On a2 of XI the male gonopore opens, and on a2 of XII the female gonopore. Beginning with V and continuing through IX, the annuli, instead of being divided by straight transverse lines as is commonly the case, are separated by corrugated or serrated lines. The three annuli of XII and about half of XIa3 are covered by the pre-urosome.

As is evident from the above somite-annuli analysis, the somites do not show a definite graduated increase as to the number of annuli comprising the respective somites. They do, however, show a constant, uniform length increase, and beginning with somite VII each somite contains a neural ganglion which is located mid-longitudinally. Because the presence of a ganglion is the fundamental test of a segment, it seems that this is of greater significance and consequently should receive greater consideration than the mere somite-annuli arrangement. Although a constant somite-annuli increase does exist in some forms (i.e., GLOSSIPHONIIDAE), a non-uniform increase is not uncommon in certain PISCICOLIDAE; viz., *Pontobdella* spp., *Trachelobdella* spp., *Stibarobdella* sp. and others.

*Urosomal Region.* The posterior region which comprises about  $\frac{3}{4}$  of the over-all length may be conveniently divided into the pre-urosome, urosome, and the posterior sucker. The sides of the body are parallel for their entire distance, with the exception that there is a slight narrowing anteriorly through the three anterior-most rings and posteriorly, beginning with XXIV, there is a more noticeable constriction, forming a peduncle to which the sucker is attached. There are twenty-two segments comprising the urosome which are divided into thirty-nine rings. Beginning



with XIIIa3 and ending with XXIV, each ring bears, on its anterior margin, a pair of lateral, broad, foliaceous branchiae which are almost half as long as the body is wide. Beginning with XIIIa2 and continuing posteriorly, eleven pairs of diaphanous respiratory vesicles are found. The first pair of vesicles occurs one annulus anterior to the first pair of branchiae, and all vesicles are located on the neural annulus, with the exception of the eleventh or last pair of vesicles. The second pair is in juxtaposition with the third pair of branchiae, the third with the sixth, and thereafter the vesicles occur in conjunction with every third pair of successive branchiae. The anus opens dorsally posterior to XXVII. The somites are of the primary or tri-annulate type, with the exception of XIII and those in the posterior region where somite-annulus diminution makes its appearance. Somite XIII is composed of two approximately equal annuli and two anterior, smaller equal rings the former two constitute a portion of the urosome, while the latter two form the pre-urosome or prepuce. The interpretation that the preputial fold is composed of two rings agrees with Apathy's interpretation (1888: 170) of the preputial fold in *Branchellion torpedinis*. However, he regarded the region in question as constituting a complete somite within itself. If one analyzes the neural annulus (the one which carries the respiratory vesicles) as the first annulus of the somite (current then) instead of the center of the tri-annulate somite (current now), *B. ravenelii* agrees exactly with *B. torpedinis* or vice versa.

Since the two rings composing the pre-urosome do not constitute a true part of a neuromere, gonomere and/or gastromere, it appears logical to consider them analogous to the first annulus of a typical tri-annulate somite, though somewhat atypical. If one considers this somite (XIII) analogous to the typical tri-annulate somite, there are ten such tri-annulate or primary somites which are followed by five comprising a lesser number of annuli. Somite XXIII is considered to be formed of two equal annuli; XXIV, XXV, XXVI, and XXVII are composed of a single annulus. The posterior sucker + one ring, which contains the posterior ganglionic mass, is assumed to represent seven somites. Therefore, the body is composed of thirty-four somites, the number composing a leech as determined by Moore and by Castle, whose conclusions were followed by subsequent workers.

The strongly developed posterior sucker, which is directed strongly ventrally, is centrally attached to the well-defined urosomal peduncle. This sucker is circular, much larger than the oral, very concave and exactly terminal, with a contracted rim in some specimens, while in others it is discoidal. On the ventral surface there exists about 400 cupules or secondary suckers.



## DIGESTIVE SYSTEM

The rather small mouth opening is situated at the center of the anterior cupuliform sucker. The proboscis ends in VIII, where it receives the numerous ducts of the conspicuous esophageal glands, which are situated some distance anterior to the point of their emptying level. The proboscis sheath consists of a tube with thick and muscular walls and covers the distance from the mouth to the level where the esophageal ducts empty. It is partly surrounded by the large anterior ganglionic mass, and on each side the esophageal glands are linearly arranged. The proboscis sheath and proboscis is an adaptation of the pharynx, which has become highly muscular, the proboscis being free, protractile and thus capable of being thrust through the small oral opening in the leech's anterior sucker into the tissues of the host.

A narrow, thin-walled tube which is called the esophagus passes posteriorly from the proboscis to somite XIV, where it expands into the stomach. The stomach possesses four pairs of single digitate expansions known as the stomach diverticula, which exist between the five pairs of testes. Between each pair of testes, the stomach is constricted so as to fall within the space between the testes comprising the pair. The intestinal cecum, which continues posteriorly to XXIII/XXIV and is placed ventrally to the intestine, has no lateral diverticula. Apparently this intestinal cecum represents two fused ceca which cohere at five places and corresponds to the fourth stage in the development of cohesion from the primitive pair of ceca, as figured by Johansson (1896, Pl. VIII, Fig. 82) for *Piscicola geometra*. The first lacuna of the intestinal cecum is contained in XVIIIa2-a3, and the three following ones are located in successive somites in the same annular position. The fifth lacuna, however, is longer and consequently is contained in the first ring of XXIII, and in a2 and a3 of XXII.

The intestine is a moderately wide, medianly situated, longitudinal portion of the alimentary canal, beginning in somite XVIIIa1 and continuing through XXIIa2. Posteriorly the canal shows a wide expansion to form the rectum, which begins at XXIIa3. Its terminal part bends sharply upwards and discharges its contents through a dorsal and median anus, situated posterior to XXVII. The intestine is characterized by four pairs of lateral posteriorly directed, recurved expansions, which are articulated into a complicated series of folds. The lateral diverticula originate anteriorly at the mid-level of each of the first four intestinal cecal lacunae—or approximately at the level of the third annulus of the respective somites.

Thus the digestive system is composed of a number of parts which, for the sake of clarity, may be designated mouth, proboscis and proboscis sheath region (pharynx), esophagus, esophageal glands, stomach with

its diverticula, intestinal cecum, intestine with its diverticula, rectum, and anus.

#### REPRODUCTIVE SYSTEM

As is true of other leeches, *Branchellion ravenelii* is monoecious and the male and female gonads are paired. The reproductive system is of great systematic value because of the great variety exhibited in the details of the various parts.

If one examines one of these animals on the ventral surface, one sees very clearly a large orifice; it is the male gonopore and is situated at XIa2. The female gonopore, a great deal smaller, cannot be clearly seen exteriorly because it is hidden by the integumentary fold which surrounds the genital region (clitellum) in the fashion of a prepuce, and is known as the pre-urosoma.

*Male Genitalia.* There are five pairs of testes disposed between XIIIa3 and XVIIIa1, lateral to the stomach and between its lateral diverticula; i.e., intersegmentally, since the diverticula occur intrasegmentally. From each testis proceeds latero-anteriorly a small ciliated duct, the vas efferens, there being as many vasa efferentia as there are testes. Laterally from the posterior testis and proceeding anteriorly on each side, is the ciliated vas deferens, which in its course receives the remaining vasa efferentia. The vasa deferentia are in the region of the testes very fine tubes little larger than the vasa efferentia, but having a larger lumen.

Anterior to the first pair of testes, in XIIIa2, each vas deferens curves slightly inward, forms a short straight loop, then continues anteriorly through the clitellar region. At the level of the male gonopore (XIa2) the vasa deferentia curve abruptly outward and continue anteriorly before expanding into the paired seminal vesicles. It is in the seminal vesicles that the spermatozoa become cemented together in compact bundles, known as spermatophores, and are stored for future use. From each seminal vesicle an ejaculatory canal continues antero-latero-ventrally, becomes thinner, and takes on several sinuities before opening to the inside and anteriorly into the well developed, spacious, glandular terminal portion, or atrium. It is muscular and evidently protrusible.

*Female Genitalia.* The female reproductive organs consist of a pair of ovaries, swollen at their posterior part, and extending posteriorly to the posterior border of XIVa1. The oviducts are slender and unite in front of XIIIa3 to form a common muscular oviductal gonopore opening in XIIa2.

A copulatory area does occur in *Branchellion ravenelii* very similar to that shown by Brumpt (1901:73) for *B. torpedinis*. In frontal and transverse sections this area is observed to begin posterior to the female gonopore and extend into the pre-urosoma.

*Cocoon Glands.* These glands—which, however, are present in almost all HIRUDINEA,—show their greatest development in the PISCICOLIDAE. The prominent ducts of these glands (clitellar glands of Bourne) always open on the clitellum and are very conspicuous. The glands extend from the posterior limit of the clitellum to the region near the anus, forming a continuous layer beneath the longitudinal muscle layers and are imbedded in the dense body parenchyma. The glands are formed of spherical cells somewhat elongated in the direction of the body length.

#### NERVOUS SYSTEM

Inasmuch as the presence of a ganglion is the fundamental test of a segment, there are thirty-four ganglia in the central nervous system. Thus, the circumpharyngeal ganglionic mass contains six, and the posterior ganglionic mass contains seven fused ganglia. Twenty-one free single ganglia lie, metamerically disposed, within the limits of the somite in the ventral chain, between the anterior and posterior masses.

The ventral, median gangliated nerve-chain with its connecting commissures runs from the base of the urosomal peduncle to V and VI where the large cephalic ganglionic mass encircles the pharynx. The anterior ganglionic mass represents six closely joined neuromeres and the posterior ganglionic mass seven.

Throughout the middle portion of the body the nerve-chain ganglia are about equal distances apart, but toward the ends they are crowded closer together.

#### SUMMARY

Material collected from the coast of Florida has rendered possible a detailed morphological study of *Branchellion ravenelii* (Girard, 1850), including for the first time the internal morphology of this species. Verrill's leech collection, in which was one specimen labeled *Phyllobranchus ravenelii* = *Branchellion ravenelii*, has been available for comparison.

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## EXPLANATION OF PLATE

A—anus	G—ganglion	PS—proboscis sheath
AT—atrium	I—intestine	PU—preurosoma
C—cupule	IC—intestinal cecum	R—rectum
CL—cecal lacuna	LB—lateral branchus	RV—respiratory vesicle
E—esophagus	LD—lateral diverticula	S—stomach
ED—esophageal gland duct	M—mouth opening	SD—stomach diverticula
EG—esophageal gland	MG—male gonopore	SV—seminal vesicle
EJ—ejaculatory duct	O—ovary	T—testis
EY—eye	OV—oviduct	VD—vas deferens
FG—female gonopore	P—proboscis	VE—vas efferens

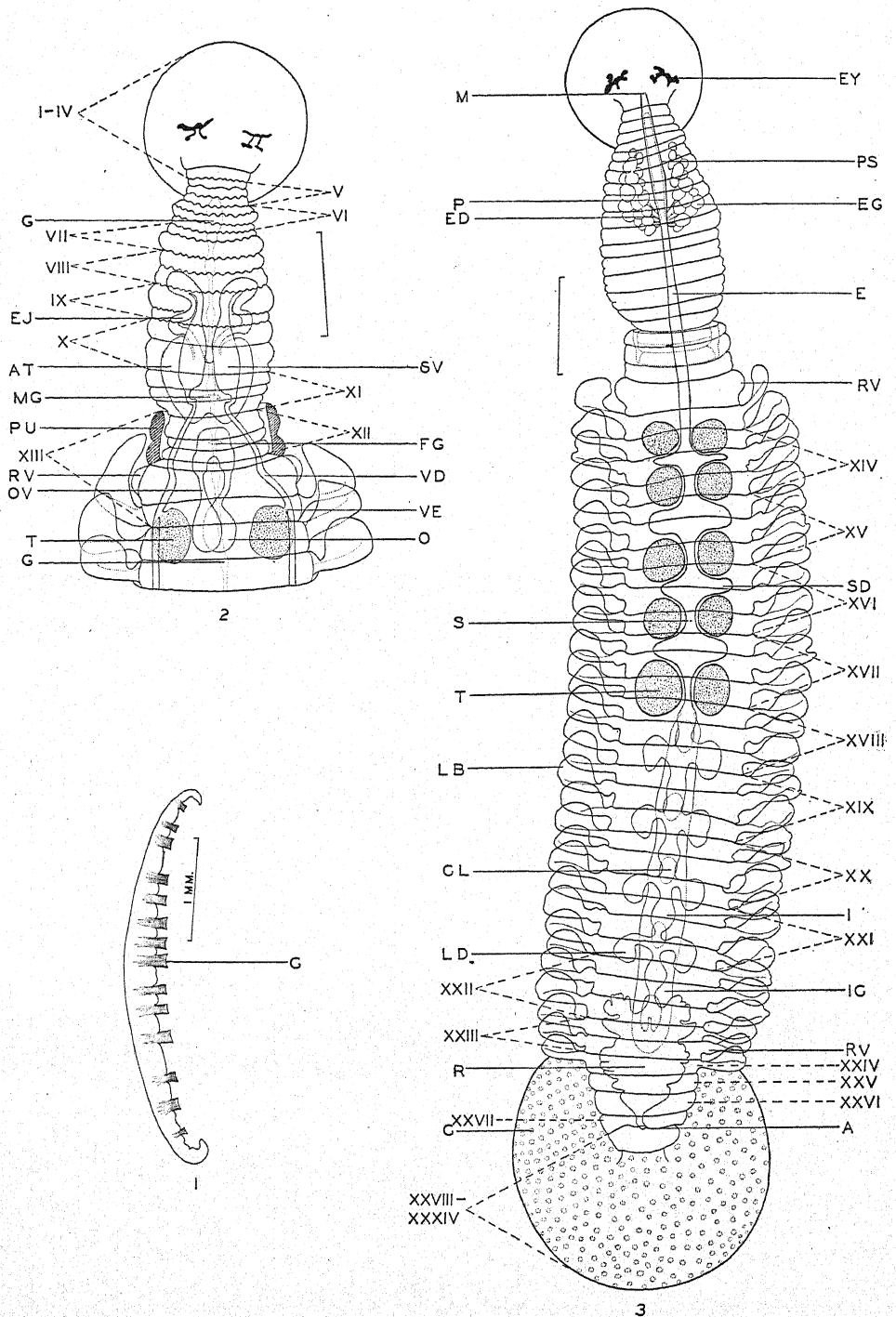
Each scale line represents 1 mm.

FIG. 1. Transverse section through the posterior sucker, showing cupules.

FIG. 2. Anterior region, showing in detail the reproductive systems and annuli-somite arrangement of the trachelosome (Dorsal view).

FIG. 3. Dorsal drawing of entire animal, showing in detail the digestive system.



*Branchellion ravenelii* (Girard, 1850)



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## STUDIES ON THE LIFE HISTORY OF THE ANOPLOCEPH- ALINE CESTODES OF HARES AND RABBITS\*

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The anoplocephaline cestodes of rabbits and hares are distributed among the genera *Andrya*, *Cittotaenia*, *Paranoplocephala* and *Schizotaenia*. Reviews of the described species were published by Baer (1927) and Arnold (1938). The latter author showed that the cestodes from leporine hosts in North America are distinct from those of Europe. His findings are not surprising, since the hares of the two continents belong to different species and the rabbits to different genera. Two species of *Andrya* have been described from European hares and rabbits and although the genus is represented in North America, no species have been reported from leporine hosts in the Western Hemisphere. Similarly *Paranoplocephala wimerosa*, described from hares and rabbits in Europe, is the only species recorded from these hosts, although additional species of the genus are known to infect other animals in different parts of the world. The genus *Schizotaenia* appears to be limited to the Western Hemisphere. Although *S. americana* occurs in hares in North America, none of the other species in either North or South America has been reported from leporine hosts. On the other hand, the genus *Cittotaenia* has representatives in both European and North American rabbits and hares: *C. ctenoides*, *C. denticulata* and *C. pectinata* in Europe and *C. variabilis*, *C. perplexa* and *C. pectinata americana* in North America. Incidence of infection with European species of *Cittotaenia* was reported by Riehm (1881), John (1926), Rees (1933a), Stunkard (1934) and Evans (1940). John gave a detailed description of *C. denticulata* and Rees of *C. pectinata*; Evans reported the incidence of the two species for each month of the year. It is interesting to note that these authors found *C. pectinata* and *C. denticulata* very common in wild rabbits of Great Britain, but did not report *C. ctenoides*. In Germany, Riehm found *C. ctenoides* the most common species in rabbits, while *C. pectinata* was rare in rabbits and common in hares. My records agree with those of Riehm.

The life history of the anoplocephaline cestodes, which for more than

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a half century has been one of the most enigmatic problems in parasitology, is now at least partially disclosed. Previous studies on the life cycle of these tapeworms were reviewed by Stunkard (1934). That paper recorded experiments conducted during the academic year 1931-1932 at the Institut für Schiffs- und Tropenkrankheiten in an attempt to discover the life cycle of the cestodes of rabbits. These animals appeared to be ideally suited for such investigation. Rabbits are small, can be maintained in close confinement, and their food can be examined more easily for infective agents than that of larger herbivores. The wild rabbits of Northern Germany are heavily infected with tapeworms and since the domestic variety was derived from the wild European rabbit and is susceptible to infection with certain of the cestodes of wild rabbits, the selected location and species afforded an opportunity to pursue the study under favorable and well controlled conditions. Results of the experiments demonstrated that the onchospheres of *Cittotaenia* complete their development in the egg and that they do not become infective for the final host. Since direct infection in these species does not take place, an intermediate host must be necessary for the completion of the cycle. From bionomic data, the characteristic features of the intermediate host were clearly delineated.

Utilizing the information acquired from the Hamburg experience, study of the life history of anoplocephaline cestodes was continued in New York and the life cycle of *Moniezia expansa* was worked out and reported (Stunkard, 1937a, 1937b, 1937c). Galumnid mites were found to serve as intermediate hosts and the results have been confirmed by Stoll (1938), Krull (1939a) and Shorb (1939). More complete accounts were presented by Stunkard (1939a, 1939b). The life cycle of a second anoplocephaline species, *Bertiella studei* a parasite of monkeys and man, was reported by Stunkard (1939c, 1940a, 1940b). The life history of a third species, *Cittotaenia ctenoides* from European rabbits, was reported by Stunkard (1939d). The present paper presents a more complete account of the development of *C. ctenoides*, and observations on the life cycle of two other species, *C. denticulata* and *C. pectinata*. So far as known, all of these anoplocephaline cestodes employ oribatid mites as intermediate hosts; consequently it becomes increasingly probable that other members of the family have a similar life cycle.

#### EXPERIMENTAL METHODS AND RESULTS

It was a great satisfaction to return to Hamburg in the summer of 1938 to continue the studies begun there seven years before. The discovery of the life cycle of *Moniezia expansa*, which indicated that oribatid mites may serve as intermediate hosts of the rabbit cestodes, and the experience of the previous year in the "Tropeninstitut" were of value in

outlining the present investigation. Wild rabbits are not readily obtained in Hamburg during the summer and early autumn. Accordingly, it seemed wise to devote this period to the collection and dissection of mites, in an effort to discover cysticercoids of natural infection. Such cysticercoids could be fed to young, laboratory-raised domestic rabbits and if the larvae developed to sexual maturity it would be possible to identify both the cestodes and their field hosts. Information concerning the natural intermediate hosts would be very helpful in planning experimental infections later in the season when wild rabbits are abundant in the markets and eggs from identified cestodes can be obtained in large numbers.

Representatives of the mites common in the Hamburg area were identified by Dr. Max Sellnick of Königsberg. Grateful acknowledgment is made for his kindness in determining the material. Although identified specimens were used for comparison, it is possible that some of the mites examined for natural infections and others used later in feeding experiments were not correctly identified, because of the rapidity with which mites were dissected and the inherent difficulty of specific determination by one who is not a specialist in the group.

Work on the life cycle of the rabbit cestodes was impeded during the fall by experiments conducted simultaneously to determine the life history of *Bertiella studeri*. In the latter studies (Stunkard, 1940a), eggs of the parasite were fed to many species of mites, collected in areas where they would not ordinarily be exposed to eggs of anoplocephaline tapeworms. The discovery that the onchospheres of *Bertiella* would emerge in the intestine of various mites and begin development in the body cavity, suggested that more than one species might be able to serve as intermediate hosts of this and other members of the family ANOPOLOCEPHALIDAE.

#### *Experimental Infection of Rabbits*

Mites were collected in the regions of Wohldorf and Duvestedt and dissected in the attempt to obtain natural infections with the cysticercoids of rabbit cestodes. Incidence of infection in mites would depend naturally on the number of rabbits in the district and on the incidence and intensity of infection among them. Consequently, mites were collected in areas where rabbit feces were abundant and yielded large numbers of cestode eggs. The collections were made after light rains or in the early morning or late evening. Larger numbers of mites could be collected on dark, cool, humid days. A moist area was selected where the grass was at least 10 cm high. With a sharp garden tool, the roots of the grass were cut 2 to 3 cm below the surface of the ground and pieces of sod 10 to 20 cm in diameter were taken. These samples of earth, grass roots and grass were placed in closed containers and transported to the labora-

tory. The material was then screened through a fine sieve on a large piece of white paper which was brightly lighted. The light stimulated the mites to move and they were picked up with a moist, camel's-hair brush. Krull (1939b) described the method employed by him for the collection of oribatid mites. Grass was cut in the field and transferred to the laboratory where the mites were washed from it by warm or cold water. Krull stated, "It appears that the actual number of mites on grass varies considerably, and that there are times, even in spring, when no mites can be recovered." He noted also, "They appear to leave the grass when there is a high wind." I have observed that mites often drop from the grass when it is disturbed and the method of Krull would probably recover only a fraction of the specimens present in an area. In the same paper Krull reported that mites can not ingest *Moniezia* eggs, but that the mite makes a hole in the shell and ingests the contents. Eggs to be accessible must be well anchored; otherwise they were merely pushed around. This observation by Krull is supported by my finding (January 14, 1939) of six pyriform apparatuses with larvae inside them but without egg shells, in the intestine of a specimen of *Liacarus coracinus* which had been fed *Cittotaenia denticulata* eggs the previous day.

Over six thousand mites, belonging to thirty-eight different species, were dissected between July 27th and November 1st. On September 8th, in a specimen of *Scutovertex minutus*, I found a cysticeroid (Fig. 25) which closely resembled that of *Moniezia expansa*. Examined in a drop of water without a coverglass, it measured 0.216 mm in width and 0.18 mm in length. The apical end was slightly flattened and there was a small fibrous mass, the remains of the cercomere, attached at the base of the cyst. The cyst wall was firm and fibrous, and calcareous bodies were loose in the cavity between it and the scolex. The scolex was 0.13 and the suckers 0.06–0.07 mm in diameter. Movement of the larva and suckers was noted. A small hole was made with a needle in a grain of wheat, the larva was placed in it and then fed to a half-grown rabbit, No. 1. The feces of the rabbit were examined twice each week. They were negative until November 24th, when seven cestode eggs were recovered in the examination of forty fresh pellets. A few eggs were found in feces collected on November 25th, and they were abundant in feces collected November 26th. The rabbit was killed on this date and a single, fully developed specimen of *C. ctenoides* was found in the intestine. It was very active, and after removal of the sixteen terminal proglottids, the worm was extended and fixed. Eleven weeks had elapsed from the time the larva was ingested until eggs were found in the feces. A brief preliminary note, (Stunkard, 1939d) announced the life cycle of *C. ctenoides*.

On September 19th, dissection of a specimen of *Scheloribates laeviga-*



*tus* yielded a cysticercoïd. It was almost spherical, 0.188 mm in diameter, was slightly flattened at the apical end and bore a very short, stalk-like cercomere. The cyst wall appeared to be a little thicker than the one found on September 8th, and there were more concretionary bodies between the larval scolex and the cyst wall. The larva was active within the cyst. The cysticercoïd was placed in a grain of wheat and fed to a half-grown rabbit, No. 2. The feces of the animal were examined twice a week for four months and no cestode eggs were found. At that time it was killed and no tapeworm was present.

On September 19th, two cysticercoïds were removed from a specimen of *Scutovertex minutus*. They were similar to the one found on September 8th, but one was slightly smaller. The cyst wall of the larger one was injured during the dissection; both were fed to the same young rabbit, No. 3. Biweekly examination of the feces of this rabbit for a four month period did not yield any cestode eggs and it was negative when dissected at that time.

Other mites from infected areas were dissected during the winter and large numbers were examined in the spring of 1939. On May 8th, a cysticercoïd which measured 0.216 by 0.2 mm was found in *Pelops tardus*. It contained an active larva and was fed to a young rabbit, No. 8. The rabbit was negative when dissected on May 30th. Since the three larvae found on September 19th failed to develop to sexual maturity and the one taken on May 8th was not recovered, their specific identity remains in question. It is probable that they were larvae of *Cittotaenia*, although deer range over the area and eggs of anoplocephaline tapeworms, probably *Moniezia*, have been recovered from the feces of deer.

#### *Experimental Infection of Mites*

The second phase of the investigation was concerned with attempts to infect mites by feeding them eggs of rabbit cestodes. In the feeding experiments, only eggs from identified cestodes were used. To secure eggs for this purpose, 20 hares and 93 rabbits were dissected during the autumn and winter. None of the hares was infected and no specimens of *C. pectinata* were found. This result was unexpected since 10 of 56 hares examined during the winter of 1931-1932 harbored *C. pectinata* and the worms were often present in large numbers, (Stunkard, 1934). Of the 93 rabbits examined, 63 yielded specimens of *C. ctenoides*, 8 contained specimens of *C. denticulata*, and 3 were infected with *Andrya cuniculi*. No mature specimens of *A. cuniculi* were found and since no eggs of *C. pectinata* or *A. cuniculi* were available, the experiments were limited to *C. ctenoides* and *C. denticulata*. Usually one or two, but never more than two mature specimens were found in a rabbit; often several immature specimens were present together with one or two ma-



ture ones. The dissection of hares and rabbits during the two seasons, 1931-1932 and 1938-1939, provided many small, immature specimens of all three species of *Cittotaenia* and made it possible to trace the successive stages of their development in the final host.

For experimental infection of intermediate hosts, mites were collected from areas to which rabbits would not have access. Representatives of all the common species were used in the experiments. The mites were maintained in culture dishes. Usually 200 to 300 mites of various species were placed in each culture. The dishes were kept at room temperature, away from direct light and provided with abundant food and moisture. When mold growth became excessive, the mites were transferred to clean dishes. Eggs of *C. ctenoides* or *C. denticulata* were added to culture dishes as recorded below. When the mites were transferred to clean dishes, which was necessary every few days at least and often daily, most of the cestode eggs were left behind. Since the cestode eggs were added to the mite cultures and allowed to remain there, it is impossible to tell when they were eaten and exact dates of infection are not available. Presumably the eggs were ingested at different times and since the larvae remain viable in the eggs for weeks in moist chambers, repeated infection probably occurred. The number of available eggs was rapidly diminished, however, by the successive transfers of the mites to clean nests. Some eggs were attached to the bodies of the mites and others were dried on pieces of filter paper and transferred with them, but most of the infections were probably acquired during the first few days. Since the object of the experiment was to complete the cycle in the intermediate host, only those mites which died in the cultures were dissected. Often when dead mites were removed and examined, they were so dried internally that larvae, if present, would have disintegrated. The larvae are exceedingly delicate and changes in osmotic relations cause them to die and decompose. Many of the larvae were photographed alive, but they were so fragile that they often disintegrated before the photograph could be made. The figures on plates I and II record successive stages in development, but blistering and other evidences of disorganization appear in the figures. The dates of exposure and dissection, names of hosts, with number and size of the larvae recovered, are presented in the following tables. Only those dissections which yielded larvae are recorded. The mites were dissected in a drop of water or Ringer's solution and sometimes two or more mites of a single species and from the same culture were dissected in a single drop. In such instances, it was not possible to determine with certainty the number of larvae which came from each mite. In the first series (Cultures I to III), begun on October 12th, the eggs came from proglottids which had been cut from a worm and the dissection of 50 mites during the first two weeks yielded

only two infections. It was feared that many of the larvae were not infective and so other eggs, from detached proglottids, were added on October 26th. With the procedure followed, it was impossible to determine precisely when the eggs were eaten and allowance for this fact must be made in the interpretation of the results.

Cultures I-III, exposed to eggs of *C. tienoides*, October 12 and 26, 1938  
Dates of dissections and results

- October 17. Two *Scutovertex minutus*: recovered 2 living onchospheres;  $0.025 \times 0.022$ , and  $0.022 \times 0.02$  mm.
- October 22. Two nymphs, *Galumna* sp.: recovered 6 larvae; largest 0.04 mm in diameter, one 0.03 mm, the others smaller.
- November 22. Three *Scheloribates laevigatus*: recovered 5 larvae; 0.065, 0.05, 0.042, 0.038, and 0.032 mm in diameter. The 3 largest ones had small cavities.
- November 24. One *Notaspis coleoptratus*: recovered 5 larvae; 0.06, 0.048, 0.041, 0.036, and 0.032 mm in diameter.
- November 26. One *Scutovertex minutus*: recovered 5 larvae; the largest (Fig. 2) was 0.095 mm in diameter, the smallest (Fig. 1) was merely an onchosphere,  $0.028 \times 0.025$  mm.  
Two *Scheloribates laevigatus*: recovered 3 larvae; 0.036 mm in diameter,  $0.037 \times 0.034$ ,  $0.022 \times 0.02$  mm.  
One *Trichoribates incisellus*: recovered 3 larvae;  $0.07 \times 0.07$ ,  $0.038 \times 0.034$ , and  $0.024 \times 0.022$  mm.
- November 28. One *Scheloribates laevigatus*: recovered 3 larvae;  $0.075 \times 0.075$ ,  $0.032 \times 0.028$ , and  $0.022 \times 0.02$  mm.
- December 5. One *Galumna obvius*: recovered 3 larvae; largest  $0.032 \times 0.029$  mm.  
One *Galumna nervosus*: recovered 2 larvae, largest  $0.037 \times 0.035$  mm.
- December 10. One *Scheloribates laevigatus*: recovered 4 larvae;  $0.15 \times 0.13$ ,  $0.136 \times 0.1$ ,  $0.1 \times 0.09$ , and  $0.028 \times 0.026$  mm.
- December 14. One *Scheloribates laevigatus*: recovered 4 larvae; largest  $0.035 \times 0.034$ , one  $0.03 \times 0.027$  mm, others smaller, size not recorded.  
One *Pelops acromius*: recovered 1 larva; a young cysticeroid, cyst  $0.2 \times 0.18$  mm, cercomere 0.25 mm long and 0.08 mm wide at the base, with hooks at the distal end of the cercomere. On application of a coverglass the cyst opened and contents were everted (Fig. 9); the sucker pads were  $0.055 \times 0.048$  mm.
- December 17. One *Galumna obvius*: recovered 14 larvae; 2 young cysticeroids, in one the cyst was 0.13 mm in diameter, the cercomere 0.32 mm long and 0.07 mm wide at the base, with hooks at the tip. The other was about the same size. One pyriform larva measured  $0.33 \times 0.18$  mm, others  $0.23 \times 0.16$  mm,  $0.21 \times 0.15$  mm,  $0.14 \times 0.13$ ,  $0.1 \times 0.1$ ; the smallest larva measured 0.065 mm in diameter. The young cysticeroids everted in Ringer's solution before a coverglass was added and all the larvae disintegrated within 30 minutes.
- December 19. One *Galumna obvius*: recovered 10 larvae; 2 young cysticeroids with cercomeres about 0.3 mm long (Figs. 12, 13, 14); the cyst of one (Fig. 14)  $0.2 \times 0.19$  mm, the other (Fig. 13)  $0.17 \times 0.17$  mm. One larva was pyriform with cercomere (Fig. 8), the body 0.31 mm long with cercomere of almost equal length, the sucker discs near the anterior end and hooks in tip of cercomere were clearly visible. Two were vermiform, one (Figs. 6, 12) 0.413 mm long and 0.18 mm in greatest width, the other  $0.4 \times 0.19$  mm. The other larvae were smaller, the smallest 0.06 mm in diameter.
- December 20. One *Galumna obvius*: recovered 10 larvae; the largest was almost identical in size and appearance with the one shown in Fig. 8, one was pyriform with the beginning of cercomere constriction, the body 0.3 mm long (Fig. 7). Two were oval,  $0.24 \times 0.18$  mm, and the other (Fig. 4)  $0.2 \times 0.16$  mm. The other larvae were smaller. One *Galumna obvius*: recovered 1 cysticeroid with small atrophied cercomere. It measured  $0.18 \times 0.17$  and was fed to a young domestic rabbit, No. 4.

- December 21. One *Galumna obvisus*: recovered 12 larvae; 6 cysticercoids 0.17–0.2 mm in diameter, all with cercomeres, some slightly longer and others shorter than the diameter of the cysts. All were fed to rabbit No. 4. There were 3 vermiform larvae, 0.28 to 0.36 mm in length, similar in appearance to Fig. 6. Three larvae were oval, 0.2 to 0.36 mm in length and corresponded in development to stages shown in Figs. 4 and 5.
- December 30. One *Galumna obvisus*: recovered 3 larvae; the largest was pyriform and without coverglass measured  $0.137 \times 0.12$  mm, the smallest was spherical and about one-half as large.
- December 31. One *Liacarus coracinus*: recovered 3 larvae; the largest had pyriform body 0.15 mm long and 0.12 mm wide, the sucker pads were visible, the cercomere was 0.24 mm long and 0.07 mm wide at the base. The other larvae were oval but blistered early and no measurements were recorded.
- January 3. One *Scutovertex minutus*: recovered 3 cysticercoids,  $0.21 \times 0.18$  mm with cercomeres. They were dried and somewhat flattened, fed to rabbit No. 4.
- January 5. One *Liacarus coracinus*: recovered 4 larvae; the largest (Fig. 5) was  $0.4 \times 0.266$  mm but shortened before the photograph was taken; note the germinal cells in the specimen. The other larvae were oval, like Fig. 4.
- January 6. One *Liacarus coracinus*: recovered 5 larvae; 3 young cysticercoids with cercomeres about as long as the diameter of the cysts, fed to rabbit No. 4. One larva was pyriform with beginnings of cercomere constriction, one was vermiform.
- January 6. One *Galumna obvisus*: recovered 8 larvae; 1 cysticercoid was fed to rabbit No. 4, 3 pyriform larvae with cercomeres like Figs. 7 and 8, 4 spherical to oval larvae without cercomeres.

Culture IV, exposed to eggs of *C. tenoides*, November 21, 1938

- November 29. *Scheloribates laevigatus*: 1 larva;  $0.031 \times 0.027$  mm.
- December 14. *Trichoribates incisellus*: 6 larvae; largest  $0.136 \times 0.12$  mm, another about the same size, 1 measured  $0.11 \times 0.1$  mm (Fig. 3, note the blister and body cavity), the smallest was  $0.026 \times 0.023$  mm.
- December 27. *Cepheus cepheiformis*: 7 larvae; largest 0.11 mm in diameter, smallest 0.07 mm in diameter, all were spherical with cavities.
- Notaspis coleoptratus*: 12 larvae; largest spherical 0.087 mm, smallest  $0.034 \times 0.03$  mm.
- Notaspis coleoptratus*: 6 larvae; largest spherical 0.08 mm, smallest  $0.032 \times 0.03$  mm.
- December 28. *Notaspis coleoptratus*: 3 larvae; the largest pyriform changed to oval  $0.13 \times 0.094$  mm, second spherical 0.087 mm, smallest 0.05 mm in diameter.
- January 2. *Scheloribates laevigatus*: 3 larvae; spherical 0.072, 0.052, and 0.042 mm.
- January 23. *Notaspis coleoptratus*: 4 larvae; 3 cysticercoids, cysts 0.14, 0.13, and 0.12 mm, with cercomeres about as long as the diameter of the cysts, 1 oval larva  $0.146 \times 0.128$  mm.
- Scheloribates laevigatus* (laboratory-raised specimen): 6 larvae; the largest vermiform 0.288 mm long  $\times$  0.1 mm wide, the second measured  $0.2 \times 0.13$  mm, the smallest was spherical 0.09 mm in diameter.
- February 8. *Liebstadia similis*: 2 cysticercoids, cysts  $0.14 \times 0.13$  mm, with cercomeres 0.1 mm long and 0.015 mm wide at the base, fed to rabbit No. 5.

Culture V, laboratory raised *Scheloribates laevigatus* exposed to eggs of *C. tenoides*, November 23, 1938

- December 2. All mites dead, dissection of 2 yielded 8 larvae, all onchospheres.

Culture VI, exposed Nov. 30, 1938 to eggs of *C. tenoides* taken from the worm raised in experimentally infected rabbit, No. 1

- December 10. *Scutovertex minutus*: 2 larvae; onchospheres.
- December 17. *Liacarus coracinus*: 3 larvae; largest spherical 0.06 mm, smallest 0.04 mm in diameter.

- December 21. *Scheloribates laevigatus* (laboratory raised specimen): 3 larvae; largest  $0.036 \times 0.034$  mm.
- January 2. *Cepheus cepheiformis*: 1 larva; spherical 0.11 mm in diameter.
- January 4. *Cepheus cepheiformis*: 6 larvae; largest spherical 0.095 mm with conspicuous cavity, smallest 0.04 mm in diameter.
- January 9. *Notaspis coleopratus*: 4 larvae; largest pyriform  $0.237 \times 0.1$  mm, smallest 0.06 mm in diameter.
- January 10. *Cepheus cepheiformis*: 2 larvae; oval, same size,  $0.18 \times 0.12$  mm.
- February 8. *Liaccus coracinus*: 1 cysticeroid  $0.155 \times 0.14$  mm, with cercomere about as long as the diameter of the cyst, fed to rabbit No. 6.
- February 10. *Xenillus tegeocranus*: 11 larvae; cysticeroids (Fig. 11) largest  $0.18 \times 0.15$ , smallest  $0.1 \times 0.1$  mm, all fed to rabbit No. 6.
- February 13. *Xenillus tegeocranus*: 9 cysticeroids, largest 0.18 mm, smallest  $0.13 \times 0.12$  mm, the specimens were dried and the cercomeres could not be measured. They were fed to rabbit No. 6.

Culture VII, exposed to eggs of *C. denticulata*, November 10, 1938

- December 12. *Xenillus tegeocranus*: 1 larva;  $0.034 \times 0.03$  mm.
- December 14. *Scutovertex minutus*: 4 larvae; spherical to oval, largest  $0.12 \times 0.11$  mm, smallest 0.06 mm in diameter.
- December 15. *Cepheus cepheiformis*: 6 larvae; largest vermiform  $0.275 \times 0.12$  mm, 1 spherical 0.135 mm with a large cavity, others smaller.
- December 17. *Trichoribates incisellus*: 2 larvae;  $0.1 \times 0.085$ , and  $0.1 \times 0.1$  mm.
- December 19. *Scutovertex minutus*: 5 larvae; 2 were pyriform  $0.18 \times 0.082$  with the beginning of cercomeres, 2 were oval  $0.09 \times 0.08$  mm, the other was oval  $0.122 \times 0.094$  mm.
- December 28. *Scheloribates laevigatus*: 1 larva; spherical 0.1 mm, large cavity.
- January 2. *Xenillus tegeocranus*: 1 larva; an onchosphere.
- January 4. *Scutovertex minutus*: 1 larva; vermiform  $0.2-0.275 \times 0.1$  mm.
- January 13. *Scutovertex minutus*: 2 larvae;  $0.11 \times 0.095$ , and  $0.09 \times 0.09$ .
- January 31. *Scutovertex minutus*: 2 larvae; a cysticeroid, cyst  $0.19 \times 0.18$  mm, with cercomere 0.1 mm long, anterior end of larva everted in Ringer's solution before it could be photographed, the other larva was vermiform 0.38 mm. long and 0.1 mm in greatest width.
- February 9. *Scutovertex minutus*: 2 cysticeroids;  $0.15 \times 0.13$  mm. One is shown in Fig. 10. Both were fed to a young rabbit, No. 7.

Culture VIII, exposed to eggs of *C. denticulata*, January 13, 1939

- January 14. *Liaccus coracinus*: 6 pyriform apparatuses were found in the intestine of the mite; they contained onchospheres but no shells were found.
- February 8. *Liaccus coracinus*: 1 larva;  $0.075 \times 0.07$  mm, cells of two sizes, 6 hooks present, larva disintegrated in a few minutes.
- February 10. *Scutovertex minutus*: 8 larvae; spherical 0.05 to 0.1 mm in diameter.
- February 17. *Scutovertex minutus*: 6 larvae; spherical 0.05 to 0.09 mm in diameter.

Culture IX, exposed to eggs of *C. denticulata*, February 4, 1939

- May 12. *Scutovertex minutus*: 2 larvae; a cysticeroid 0.2 mm in diameter with a shriveled cercomere, fed to rabbit No. 9; also a pyriform larva, body 0.216 mm long and 0.086 mm wide with a cercomere 0.1 mm. long.

Development of *C. ctenoides* was observed in eleven species of mites and cysticeroids were recovered from seven different species. About ten weeks are required for the larvae to reach the cystic stage in a mite. Since development appeared to be progressing normally in other mites which became infected but died, it is possible that these larvae might have



completed their development if their hosts had lived long enough. Development of *C. denticulata* occurred in six of the eleven species, although only *Scutovertex minutus* lived long enough for cysticercoids to be produced. It is noteworthy that infections were obtained only in oribatid mites. Present results indicate that these mites are the natural hosts of the anoplocephaline tapeworms, and that the host specificity is not at all rigid.

None of the rabbits, fed cysticercoids from experimentally infected mites, passed cestode eggs in the feces and all were negative when sacrificed and examined later. It is probable that the larvae, when ingested, were not yet infective. A further possibility exists, viz., that the domestic rabbit is a less favorable host than the wild variety. This explanation is suggested by the fact that only one of five cysticercoids from naturally infected mites developed in domestic rabbits. The explanation is not supported, however, by certain other experiments. Krull (1939a) fed five cysticercoids of *Moniezia expansa* to a lamb which on post-mortem examination yielded only two worms. Shorb (1939) recovered only 52 worms from the feeding of 468 cysticercoids of *M. expansa*. Indeed, one of the lambs, which received 203 cysticercoids, apparently did not become infected. It is possible that, in the natural hosts, only a few of the ingested cysticercoids develop to sexual maturity. An observation of Krull (1939a) bears on the question of susceptibility of the domestic rabbit. He reported that three of five rabbits became infected with species of *Cittotaenia* as a result of being kept on a plot near the shore of a pond where the mite *Galumna emarginata* was present; each infected rabbit harbored two immature tapeworms. This report confirms that of Honess (1935) who infected the domestic rabbit by feeding grass from an area frequented by wild rabbits.

#### DESCRIPTION OF STAGES IN DEVELOPMENT

Specific descriptions of *C. ctenoides*, *C. denticulata* and *C. pectinata* have been presented by Arnold (1938) and previous authors, so further details concerning the sexually mature worms are not essential in the present paper.

The eggs of *C. pectinata* were described by Rees (1933a) and the eggs and onchospheres of other European species by Stunkard (1934). In that report Stunkard noted that the eggs of *C. pectinata*, *C. denticulata* and *C. ctenoides* vary in size and appearance, depending on their condition at the time of examination, and that it had been impossible to find differential features which could be used to identify eggs in the absence of records. In the present study, eggs of only *C. ctenoides* and *C. denticulata* have been available. Although their size ranges overlap, eggs of *C. denticulata* average somewhat smaller than those of *C. ctenoides*;



furthermore as a rule, in fresh eggs of *C. denticulata* the horns of the pyriform apparatus are shorter, they taper more rapidly, and more frequently the tips are crossed. In both species, the appearance of the eggs changes markedly as the shell is formed and consolidated. These features are represented in Figs. 15 and 16.

To secure cestode eggs for the experimental infection of mites, gravid proglottids were sometimes removed from entire worms, but often the terminal segments contained few or only immature eggs. Such eggs were usually irregular in shape and had either no shell or one that was soft and hyaline. Although they contained motile onchospheres, it is doubtful whether they were infective. To obtain more mature eggs, the intestines of rabbits were examined throughout their length and often single or groups of detached proglottids were taken from the cecum and large intestine. These proglottids were sometimes full of shelled eggs and occasionally the eggs had been shed from them. Since mature specimens of more than one species of *Cittotaenia* were never encountered in the same intestine, there was no possibility for mistaken identity of detached proglottids. When gravid segments were pressed, the eggs poured out on both sides. They were embedded in the uterine secretion which adhered in whitish strings, vermiform in appearance, and as much as 5 mm in length. No preformed uterine pores, as described by Baylis (1934) for certain anoplocephaline species, have been observed in *Cittotaenia*; on the contrary, the lateral walls of the proglottids disintegrate and rupture as described by Stunkard (1940a) in *Bertiella studeri*. In water or after alternate drying and moistening, the eggs slowly separated and the shells became more compact.

In their early stages, eggs of *C. ctenoides* have no shell; they are bounded by a membrane, are spherical to oval with flattened areas where they have been compressed, and measure from 0.05 to 0.09 mm in diameter. There is an eccentric vesicle, 0.025 to 0.035 mm in diameter, attached to the external membrane and suspended in a fluid matrix. This matrix contains small granules or globules which manifest Brownian movement. The vesicle contains an onchosphere, the hooks of which may move slowly. This vesicle transforms into the cornuate pyriform apparatus, and a shell is deposited later on the external membrane. The shell is formed from the uterine secretion and at one stage appears thin and granular. At other times it consists of a series of transparent concentric rings. Such an egg is shown in Fig. 15. Older eggs have firm, hard shells, as described by Stunkard (1934). Although there may be larger and smaller ones, most of the eggs fall in the size range 0.075 to 0.085 mm in diameter. The pyriform apparatus is almost as long as the diameter of the egg, 0.021–0.025 mm wide at the base, with uniformly tapering horns. The onchospheres measure 0.019–0.021 mm in diameter

and their hooks are 0.010–0.011 mm long. Like those of *C. ctenoides*, young eggs of *C. denticulata* have no shell; indeed, eggs removed from proglottids seldom have shells. The outer covering of the egg is membranous, and the eggs, enclosed in a jelly-like mass, are arranged in irregular rows. The jelly mass is extruded with the eggs and later splits between them, contributing to the formation of the shell. Each egg is situated in a polyhedral mass which later shrinks and tends to become spherical. Usually the proglottids are shed before the eggs are shelled, but they are probably infective at this time. On November 10, 1938, a group of three proglottids was removed from the terminal part of the large intestine of a rabbit. The eggs pressed from them had only membranous coverings and were embedded in the jelly-like substance. These eggs were fed to mites in the experiment recorded earlier (Culture VII) and produced infections, although it is possible that they aged somewhat in the moist chambers before they were eaten.

No constant differences were noted between the onchospheres of *C. ctenoides* and *C. denticulata*. The experiments demonstrate that the larvae of both species may begin development in any one of a number of different oribatid mites and it is probable that development may be completed in more than one species of intermediate host. Early developmental stages of the two species are indistinguishable, and so only one species is represented in the figures of Plate I. Development of *Citotænia* in the intermediate host is very similar to that of *Moniezia* as described by Stunkard (1939a).

The onchospheres are active in the body cavity of the mite for two or three weeks. At the time of infection each onchosphere contains ten to fourteen large, germinal cells. In the haemocoel of the mite, the germinal cells multiply and the larva increases in size. Meanwhile the organization of the onchosphere is lost, its muscles atrophy, the hooks lose their regular positions and become functionless, and most of the mesenchymal cells disintegrate. The larva loses its bilateral character and becomes spherical and immobile. It consists of a mass of cells with an irregularly-shaped cavity on the side which bears the hooks. Such spherical larvae (Figs. 2, 3, 18, 19) measure from 0.04 to 0.11 mm in diameter and about four weeks are required to reach the larger size. The spherical stage is followed by one in which the larva becomes elongate and oval or pyriform (Figs. 4, 5, 20). As development proceeds the larvae become more elongate and vermiform, calcareous bodies appear in the parenchyma, muscles differentiate in the body wall and faint uncoordinated movements are discernible (Figs. 6, 21). The end opposite the hooks is much wider than the other and, with further development of the muscles, movements become more regular and contraction of the circular fibers may produce a segmented appearance. Such larvae may

extend to a length of 0.4 mm. Later a definite zone of contraction cuts off a cercomere portion from the body proper (Fig. 7). This process continues until a condition represented by Fig. 8 is reached. At this stage the rudiments of the suckers are present as lenticular, cellular aggregates (Figs. 8, 22) and the posterior end of the body contains a cavity or a mass of spongy, vesicular tissue. The anterior end of the larva, which contains the sucker rudiments, is then retracted and the sides close over it to form the wall of the cyst. At first the closure is incomplete and slight pressure on the cyst causes the contents to be everted (Fig. 9). When the cyst is formed, the cercomere is longer than the diameter of the cyst and some of the hooks of the onchosphere can usually be found at or near the posterior end of the cercomere. With further development, the scolex of the cestode is differentiated and the suckers acquire a cup-shaped form. The cercomere shrivels, and in the cysticercoids removed from naturally infected mites, it was merely a fibrous appendage to the cyst. There is much evidence to show that the rate of development in the intermediate host is highly variable. Many factors probably contribute to this result; the species of mite, number of larvae present in the haemocoel, relative age and degree of development of particular larvae, etc. This subject was discussed in the paper on the development of *Moniezia* in the intermediate host (Stunkard, 1939a), and the same factors probably operate in both *Moniezia* and *Cittotaenia*. The time required for the cysticercoid to complete its development and become infective is unknown, but probably three or four months is the usual period. The rate is undoubtedly affected by temperature, and development is more rapid during the summer months.

The taenioid cestodes have two larval stages in the life cycle, each infective for the appropriate consecutive host. The onchosphere completes its development in the egg and when released this larva progresses with the hooks in advance. The vermiform larva, which is formed by multiplication of germinal cells present in the onchosphere and which develops into the cysticercoid, moves with the other end forward, i.e., the one opposite the larval hooks. There is, accordingly, a metamorphosis between the two larval stages, a reorganization which involves replacement of the cellular elements, and a reversal of the anteroposterior axis of the larva. The vestiges of the onchosphere together with the hooks are cut off in the degenerating cercomere at the posterior end of the second larva. The scolex, which develops from the anterior end of the second larva, remains the physiological anterior end of the definitive cestode.

It is apparent that rabbits acquire the infection by accidentally eating mites which contain cysticercoids. About eleven weeks are required for the development of *C. tenoides* in the final host, i.e., from the ingestion

of the cysticeroid until the appearance of eggs in the feces. Pertinent data concerning the similar period in the development of *Andrya cuniculi* were reported by Stunkard (1934). A rabbit taken on April 24th, 1932, and brought to the animal house on April 28th was dissected on May 18th and contained a small, sexually immature specimen which bore the terminal proglottid. Another rabbit caught at the same time began to pass eggs of *A. cuniculi* on July 1st, 67 days after it was captured. Since there was no opportunity for infection in the animal house, the animal must have ingested the parasite at least ten weeks before eggs were passed in the feces.

The development of *C. ctenoides*, *C. denticulata* and *C. pectinata* in their final hosts has been traced by the removal of young worms from the intestines of naturally infected rabbits and hares. A selected series, illustrating the growth of each species is presented in Plates IV and V. The identification of the worms was made by tracing back successively younger stages from ones which were readily identifiable. Differences in size of scolices and suckers, together with relative development of proglottids and reproductive organs, were used for specific determination. The photographs were made from fixed and stained specimens and the size of each is given in the explanation of the figure.

Evans (1940) referred to reports by various authors who found specimens of *Cittotaenia* in the body cavity of rabbits. He stated, "These continued records of species of *Cittotaenia* being found in the body cavity are interesting, since they support the contention of Stoll (1937) that *Moniezia expansa* migrates in the body of the sheep during the earliest part of its life in the host." In commenting on the suggestion of Stoll, Stunkard (1939a) pointed out that it is doubtful to what extent his data support the hypothesis of a preliminary tissue invasive stage of *M. expansa* in sheep.

Like Evans and others, I have often found specimens of *Cittotaenia* in the body cavity of rabbits and hares that had been shot in the field and examined some time later. An observation by Riehm (1881) has a significant bearing on this subject. Referring to *C. pectinata* he stated, "Sie bewohnen meist den vorderen Abschnitt des Dünndarmes, nahe am Magen, doch darf man es auch nicht unterlassen, in der Leibeshöhle, namentlich zwischen den Lappen der Leber nach ihnen zu suchen, weil sie häufig genug durch die mörderische Schrotkugel aus ihrem eigentlichen Wohnsitze herausgerissen werden oder auch durch eine auf diesem Wege entstandene Öffnung in der Darmwandung nach dem Erkalten ihres Wirtes herauskriechen." My experience agrees with that of Riehm; I have never found a cestode in the body cavity of a rabbit that had not been shot and know of no record of such an occurrence. Observation of abnormal and post-mortem conditions may easily lead to unwarranted



conclusions. After the host has been dead for several hours and degenerative changes have begun in the intestine, the worms migrate through any available opening but, so far as I am aware, specimens of *Cittotaenia* do not pass through the intestinal wall of live and healthy rabbits.

Even if older cestodes normally leave the intestine, which is very unlikely, it does not follow that the very young worms do so. The idea that specimens of *Cittotaenia* undergo an early developmental phase in the tissue or in the body cavity of the rabbit finds no support from the present investigation. The cysticercoid of *C. ctenoides* (Fig. 25) which developed in a domestic rabbit, contained a scolex 0.13 mm in width with suckers 0.06–0.07 mm in diameter. In a young worm of the same species, which when permanently mounted measured 1.1 mm in length (Fig. 34), the scolex was 0.165 mm in width and the suckers 0.069 mm in diameter. Some shrinkage undoubtedly resulted from the fixation and mounting of the young worm, but the small difference in size of scolex and suckers indicates that the specimen had not passed through a developmental stage in the tissue or body cavity of the rabbit. Furthermore, the smallest specimen of *C. denticulata* removed from the intestine (Fig. 26) is only 0.3 mm in length and 0.16 mm in width. It is only slightly larger than the scolex of the cysticercoid. There appears little possibility that it had developed in some extra-intestinal location. Indeed, the finding in the intestines of rabbits, of more than 200 specimens of *Cittotaenia* which were less than 10 mm in length, negates the hypothesis that the young worms have a developmental stage during which they leave the intestine. It is probable that the scolices, after liberation from the cysts, attach firmly to the intestinal wall and between the villi, but no critical evidence has so far been submitted to demonstrate a tissue invasive or peritoneal stage in their development.

#### ABSTRACT SUMMARY

Thousands of free-living mites were collected near Hamburg from areas where wild rabbits were numerous and heavily infected with anoplocephaline cestodes. The mites were dissected and cysticercoids were removed from *Scutovertex minutus*, *Scheloribates laevigatus* and *Pelops tardus*. The larvae were fed to young, domestic rabbits and a cysticercoid from *S. minutus* developed into a sexually mature specimen of *Cittotaenia ctenoides*. Other mites, collected from areas where they would not be exposed to eggs of anoplocephaline cestodes, were fed eggs of *Cittotaenia ctenoides* and *C. denticulata*. Developmental stages of *C. ctenoides* were recovered from the body cavities of *Scutovertex minutus*, *Galumna obivious*, *Pelops acromius*, *Liaccarus coracinus*, *Notaspis coleoptratus*, *Liebstadia similis*, *Xenillus tegeocranus*, *Scheloribates laevigatus*, *Cepheus cepheiformis*, *Trichoribates incisellus*, and *Galumna*



*nervosus*. The larvae attained the cysticeroid stage in the first seven of the species listed and, since development appeared normal in the other mites, it is possible that they might have completed their development if the hosts had lived long enough. Development of *C. denticulata* was observed in *S. minutus*, *X. tegeocranus*, *C. cepheiformis*, *T. incisellus*, *S. laevigatus*, and *L. coracinus*, although only *S. minutus* lived long enough in the cultures for cysticeroids to be produced. Infections were obtained only in oribatid mites. None of the rabbits fed cysticeroids from experimentally infected mites gave evidence of infection and it is probable that these larvae were not entirely mature. The developmental stages of *C. ctenoides* and *C. denticulata* in the intermediate hosts are described from experimental infections. The development of *C. ctenoides*, *C. denticulata* and *C. pectinata* in the final hosts is described from natural infections. No evidence was found to support the idea that these tapeworms migrate from the intestine to the body cavity of normal, live, rabbits.

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## EXPLANATION OF PLATES

## PLATE I

Unretouched photographs of living precystic stages in the development of *C. ctenoides*. All specimens were removed from experimentally infected mites. Details concerning dates of exposure and dissection are presented in the text.

FIG. 1. Onchosphere,  $0.025 \times 0.028$  mm, from *Scutovertex minutus*.

FIG. 2. Spherical larva, 0.095 mm in diameter, from *S. minutus*.

FIG. 3. Spherical larva,  $0.11 \times 0.1$  mm, from *Trichoribates incisellus*; note the cavity and blistered surface.

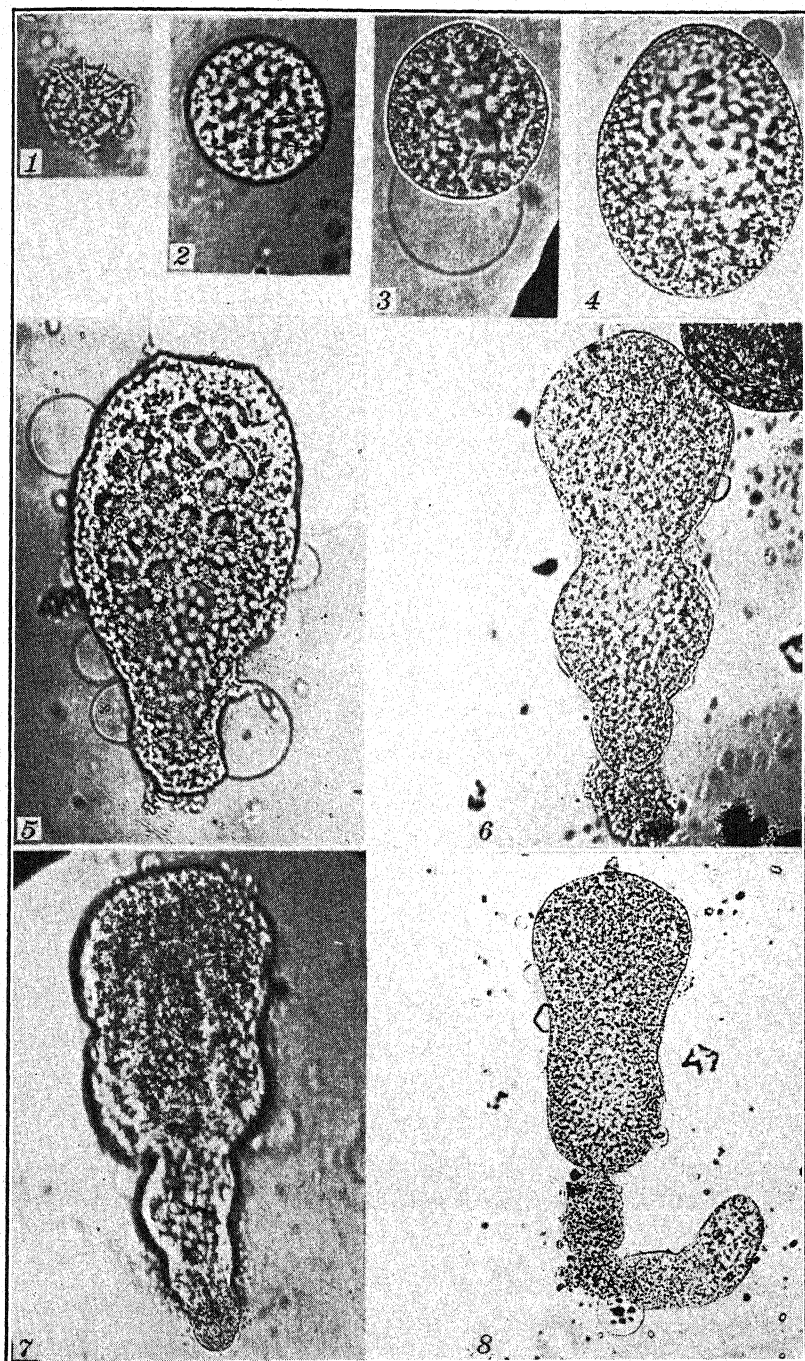
FIG. 4. Oval larva,  $0.2 \times 0.16$  mm, from *Galumna obviou*s; note the large cavity.

FIG. 5. Pyriform larva,  $0.36 \times 0.26$  mm, from *Liacarus coracinus*; note the large germinal cells.

FIG. 6. Vermiform larva,  $0.413 \times 0.18$  mm, from *G. obviou*s.

FIG. 7. Larva with beginning of cercomere formation, body 0.3 mm long, from *G. obviou*s.

FIG. 8. Pyriform larva with completely formed cercomere, body 0.31 mm long, from *G. obviou*s, note the rudimentary sucker pads near the anterior and the loose tissue near the posterior end of the body.





## PLATE II

Unretouched photographs of living postcystic stages in the development of *C. ctenoides* and *C. denticulata*. All specimens were removed from experimentally infected mites. Details concerning dates of exposure and dissection are presented in the text.

FIG. 9. *C. ctenoides*, young cysticeroid from *Pelops acromius*, cyst  $0.2 \times 0.18$  mm; contents of cyst extruded on the application of a coverglass.

FIG. 10. *C. denticulata*, cysticeroid from *Scutovertex minutus*, cyst  $0.15 \times 0.13$  mm; note the coiled cercomere.

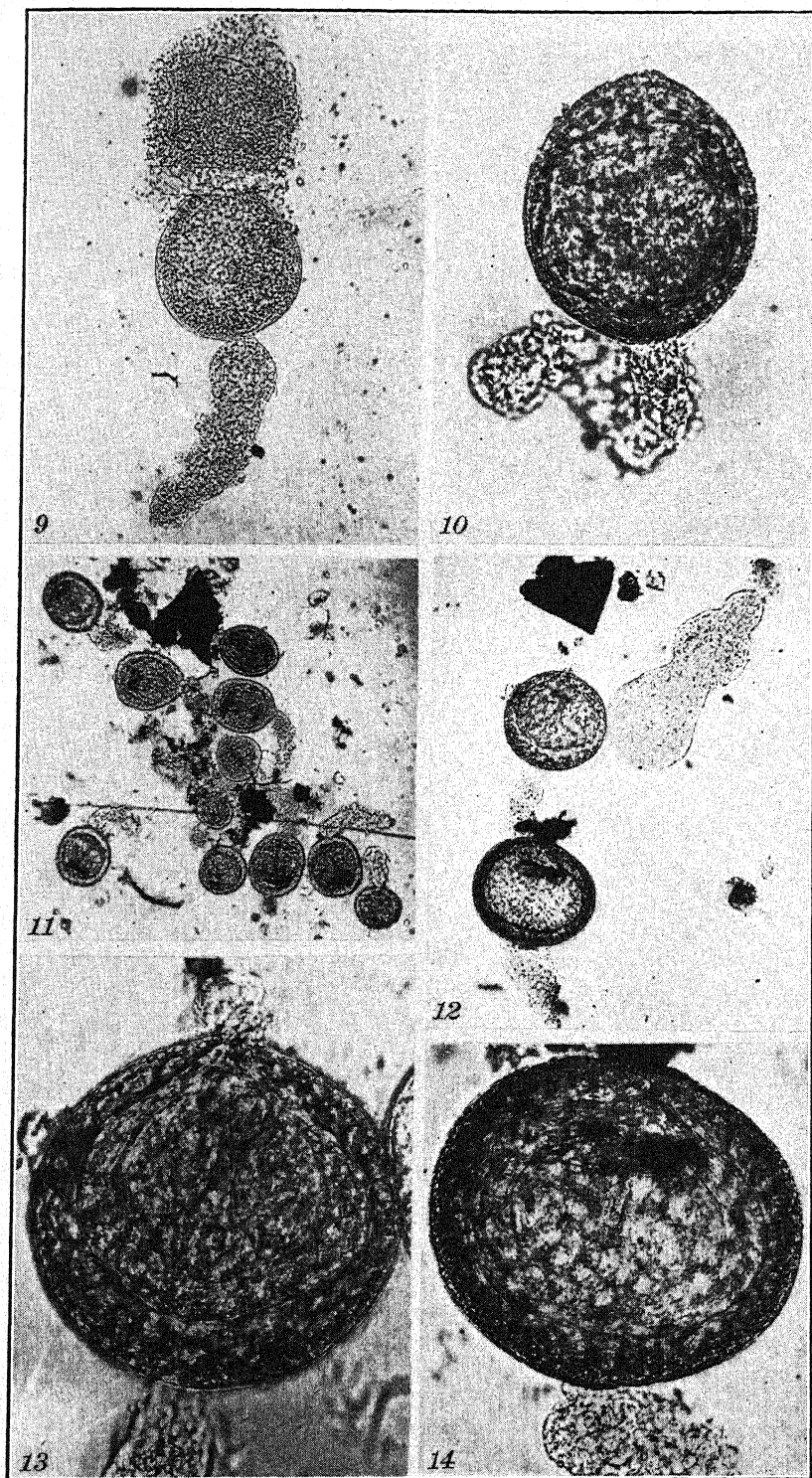
FIG. 11. *C. ctenoides*, eleven cysticeroids, all from a single specimen of *Xenillus tegeocranus*, largest cyst  $0.18 \times 0.15$  mm.

FIG. 12. *C. ctenoides*, two cysticeroids and one vermiform larva from *Galumna obvia*. The vermiform larva is shown more highly magnified in Fig. 6. The cysticeroid adjacent to the vermiform larva is shown in Fig. 13, the other in Fig. 14.

FIG. 13. *C. ctenoides*, cysticeroid,  $0.17 \times 0.17$  mm; evaporation of water and pressure of the coverglass has caused the emergence of a little material at the apex of the cyst.

FIG. 14. *C. ctenoides*, cysticeroid,  $0.2 \times 0.19$  mm.





## PLATE III

Free-hand drawings of eggs and larvae of *C. tenoides* and *C. denticulata*. The rate of development is variable and the ages given are estimated.

FIG. 15. *C. tenoides*, egg with hyaline shell, from an attached proglottid.

FIG. 16. *C. denticulata*, egg from a moist chamber, several days after removal from a detached proglottid.

FIG. 17. *C. tenoides*, onchosphere from body cavity of a mite, 1-2 weeks development.

FIG. 18. *C. tenoides*, spherical larva, 2-3 weeks development.

FIG. 19. *C. tenoides*, spherical larva, 4-5 weeks development.

FIG. 20. *C. tenoides*, pyriform larva, 6-8 weeks development.

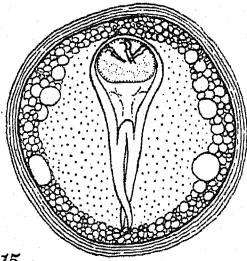
FIG. 21. *C. tenoides*, vermiform larva, 8-10 weeks development.

FIG. 22. *C. tenoides*, pyriform larva with cercomere, 10-12 weeks development; drawing based on specimen shown in Fig. 8.

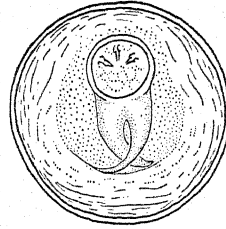
FIG. 23. *C. tenoides*, young cysticeroid with long cercomere, 10-12 weeks development.

FIG. 24. *C. denticulata*, cysticeroid with cercomere; drawing based on sketches and photograph of specimen shown in Fig. 10.

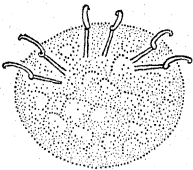
FIG. 25. *C. tenoides*, cysticeroid, drawn from pencil sketches of specimen, removed from a naturally infected *Scutovertex minutus*, which developed into a sexually mature cestode in the intestine of a domestic rabbit.



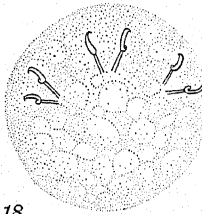
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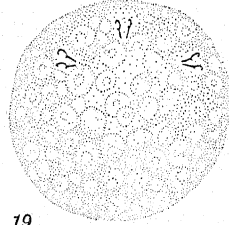
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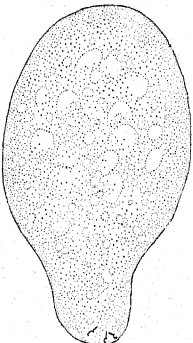
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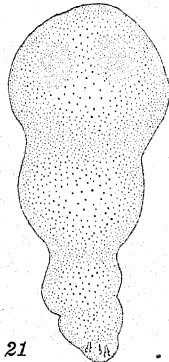
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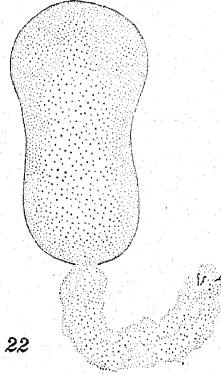
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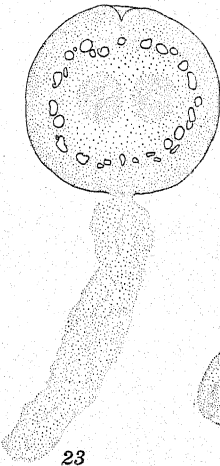
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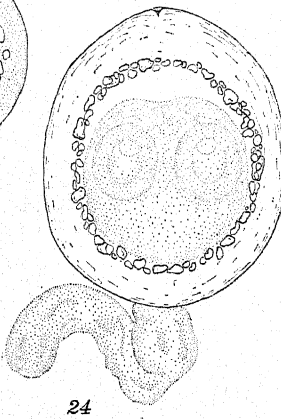
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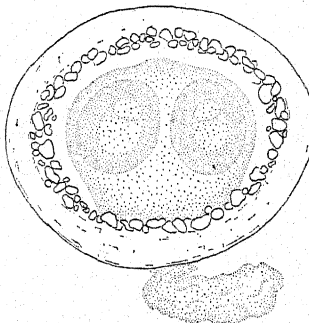
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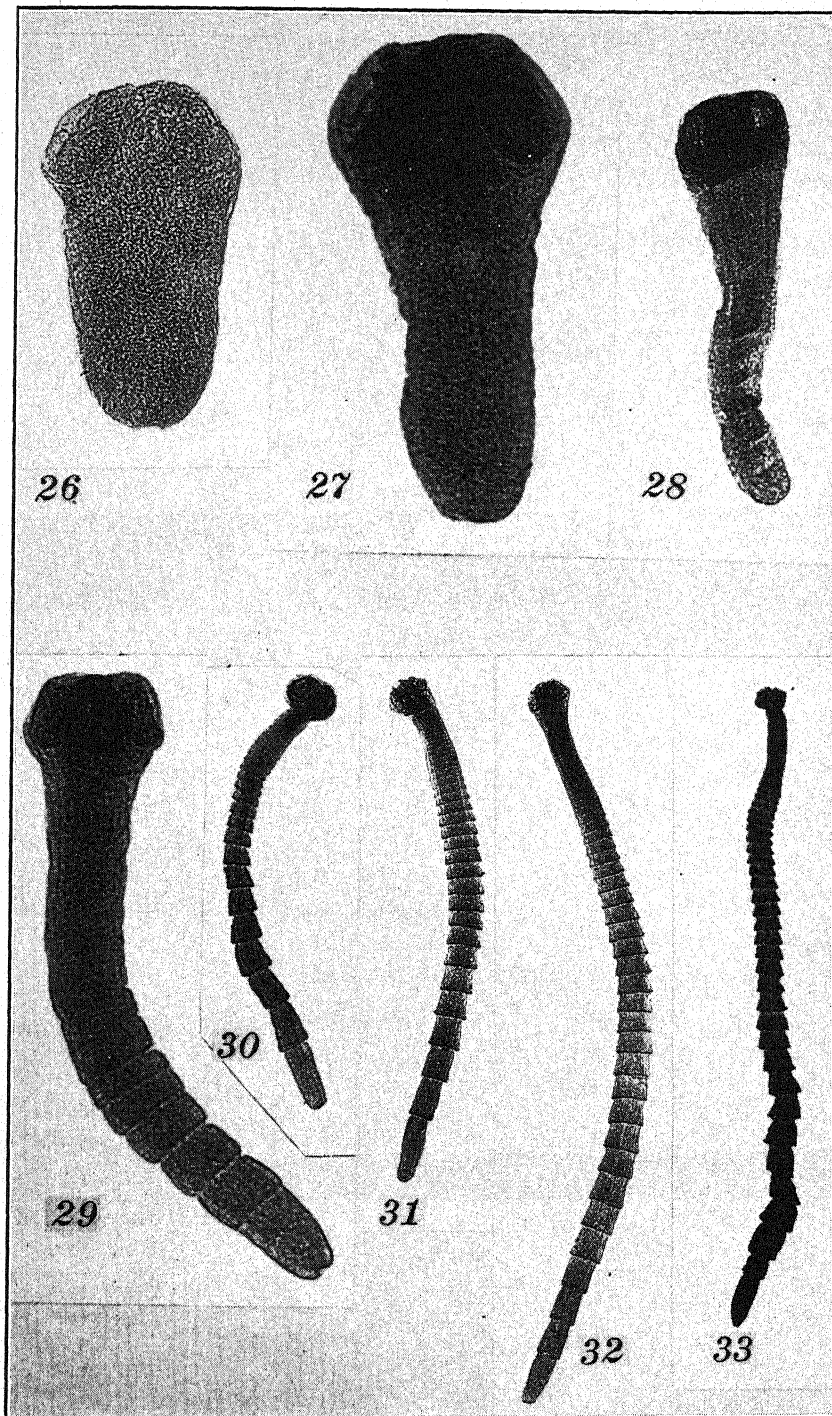
## PLATE IV

Unretouched photographs of young stages of *C. denticulata*, made from fixed and stained preparations. Actual size of the specimens is given, in millimeters.

	Length of specimen	Diameter of scolex	Size of suckers
FIG. 26.	0.3	0.16	0.07 × 0.055
FIG. 27.	0.414	0.21	0.108 × 0.08
FIG. 28.	0.885	0.26	0.13 × 0.1
FIG. 29.	1.46	0.288	0.14 × 0.11
FIG. 30.	3.2	0.38	0.18 × 0.16
FIG. 31.	5.5	0.46	0.216 × 0.18
FIG. 32.	8.3	0.5	0.24 × 0.2
FIG. 33.	12.	0.54	0.25 × 0.24

Under size of suckers, the larger measurement is the diameter and the smaller one is the depth of the sucker. In mature specimens the scolex measures 0.5 to 0.9 mm and the suckers 0.2 to 0.3 mm. The scolex, therefore, attains its growth early.







## PLATE V

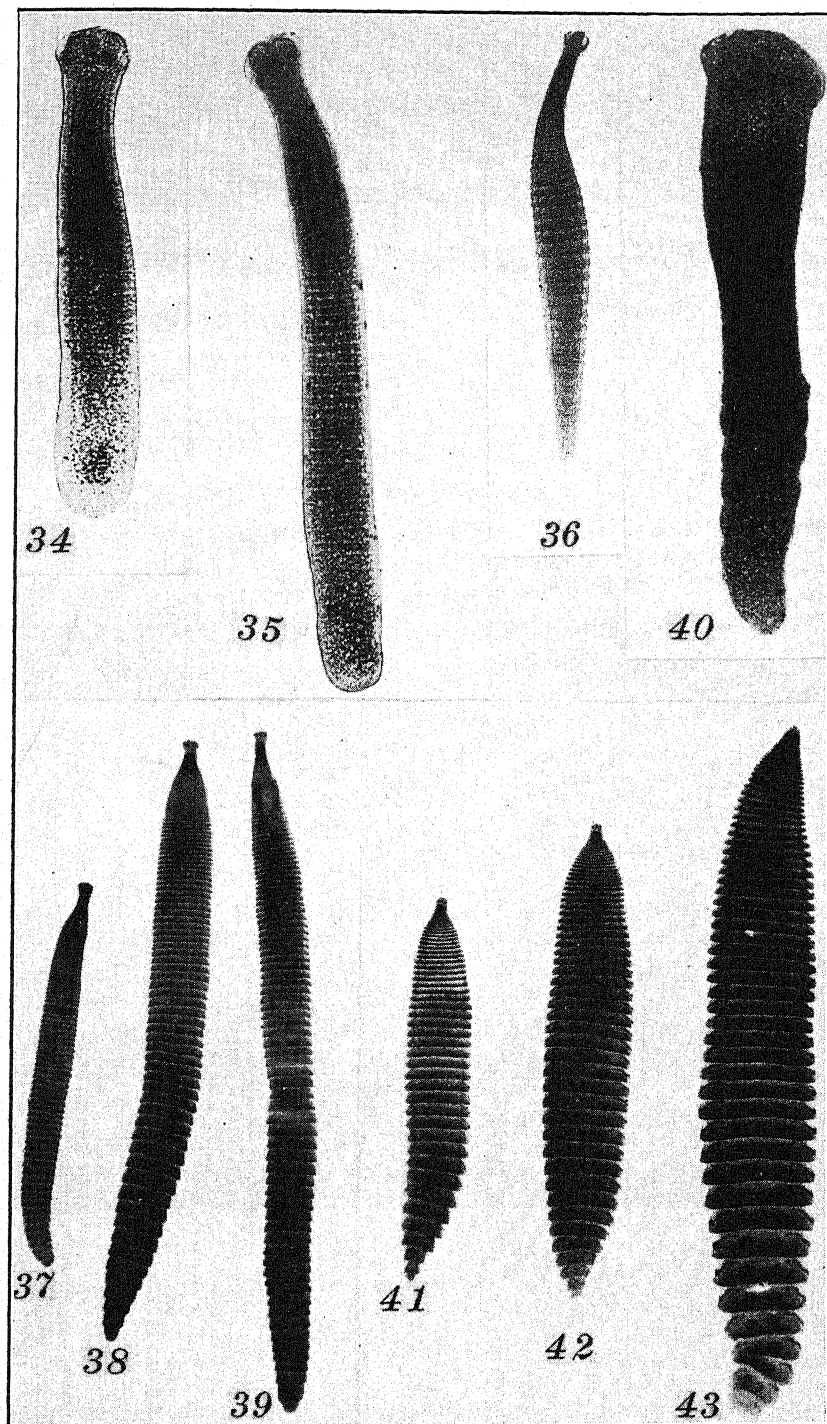
Unretouched photographs of young stages of *C. ctenoides* and *C. pectinata*, made from fixed and stained preparations. Actual size of the specimens is given, in millimeters.

	Length of specimen	Diameter of scolex	Size of suckers
FIG. 34. <i>C. ctenoides</i>	1.1	0.165	0.069 × 0.055
FIG. 35. <i>C. ctenoides</i>	1.9	0.2	0.09 × 0.072
FIG. 36. <i>C. ctenoides</i>	4.6	0.28	0.144 × 0.12
FIG. 37. <i>C. ctenoides</i>	8.2	0.316	0.158 × 0.14
FIG. 38. <i>C. ctenoides</i>	13.2	0.36	0.16 × 0.16
FIG. 39. <i>C. ctenoides</i>	15.	0.38	0.166 × 0.16

In mature specimens the scolex measures 0.35 to 0.5 mm in diameter and the suckers 0.15 to 0.24 mm.

FIG. 40. <i>C. pectinata</i>	0.97	0.2 (flattened)	0.075 × 0.055
FIG. 41. <i>C. pectinata</i>	9.5	0.25	0.128 × 0.11
FIG. 42. <i>C. pectinata</i>	11.8	0.28	0.14 × 0.14
FIG. 43. <i>C. pectinata</i>	17.	0.28	0.14 × 0.14

The scolex in Fig. 42 has reached the normal size and little if any growth is made later.





# STUDIES ON THE MORPHOLOGY OF THE *E. HISTOLYTICA*-LIKE AMOEBAE FOUND IN MONKEYS

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With the advent of the present century, protozoologists began to evidence interest in the protozoa to be found in primates other than man. In the steady accumulation of literature, the amoebic organisms found in monkeys have received much attention, mainly because of their apparently close relationship to the endamoebae in human hosts. Much has already been published in attempts to demonstrate the similarity or dissimilarity between the intestinal endamoebae in man and those in monkeys, which two hosts are, in themselves, of close evolutionary relationship.

Investigators who first encountered amoebae in monkeys often erected new species names without adequate distinction from those already known in man. Since then, however, there has evolved the increasingly accepted belief that the endamoebae of man and monkey are identical, and recent literature divides the endamoebae of monkeys into those similar, or identical, to *Endamoeba coli* in man, and those similar, or identical, to *Endamoeba histolytica* in man.

This paper will attempt to establish that the amoebae of monkeys, with specific reference to the *E. histolytica*-like type, are *not* all identical with those in man. The writer has employed both qualitative and quantitative tests of more precise nature than heretofore employed, which, it is believed, will reveal more clearly the morphological differences among the *E. histolytica*-like amoebae parasitic in monkeys.

## MATERIALS AND METHODS

Prepared slides containing *Endamoeba histolytica* from six human hosts, as well as the slides from the monkeys, were furnished by Dr. Wenrich. *E. histolytica*-like amoebae were studied from fecal smears of twelve different kinds of monkeys, involving seventeen individual hosts, whose names and sources are given in Table 3. Since the nomenclature of apes and monkeys is still in a highly confused state, the system adopted by Stiles and Nolan (1929) has been employed.

Slides fixed in Schaudinn's fluid, including five per cent of acetic acid, and stained with Heidenhain's haematoxylin, with iron alum dif-

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\* The author gratefully thanks Dr. D. H. Wenrich, under whose guidance this work has been done, both for the excellently prepared material provided by him, and for the instructive advice and suggestions received from him. Thanks are due also to Mr. Joseph Bender for his care in preparing the graphs.



ferentiation, have been mainly used. A few slides from the monkeys had been prepared by other methods.

The mensurative procedures are similar to those used by Dobell and Jepps (1918) in their study upon size races of *Endamoeba histolytica* cysts from man. As these investigators found, few of the many cysts measured were perfectly circular in profile. Therefore, most of the recorded dimensions of cysts were the mean of the shorter and longer diameters of subcircular organisms. Cysts markedly deformed in shape, infrequently seen, have not been included. In reality, cyst measurements were of the stained cytoplasmic contents within the cyst wall. As Dobell and Jepps have pointed out, the balsam mounting renders the cyst wall too indistinct for inclusion in the measurement of the cyst. Therefore, measurements do not include the cyst wall which is approximately half a micron thick, as noted by the above authors.

Although the physical handicaps in measurements have been considerable, all dimensions of nuclei have been ascertained with the greatest possible care in each case. The mean diameter of nuclei in material fixed by Yocum's picromercuric was found to be slightly greater than in material fixed with Schaudinn's fluid, but these dimensions were not used. Degenerate and obviously hypertrophied nuclei have not been measured, but these were very infrequent among the many observed.

Observations were made with a  $10\times$  ocular and a 2 mm, 1.30 N A oil immersion objective. For measurements a calibrated ocular micrometer, with a value of  $0.94\ \mu$  for each subdivision, was utilized.

Through a conversion table, measurements were converted directly into micron values. Where the diameter of the cyst or nucleus did not lend itself to definitive measurement, the measurement was made to the nearest micrometer unit. By this method, the maximum personal observational error was kept as close as possible to plus or minus half a micron, a spread of one micron or one micrometer division. This agrees with the findings of Dobell and Jepps, which demonstrated that, with very careful measurement, "the individual error would not be greater than one unit of the scale employed."

#### *Endamoeba histolytica* Schaudinn

This species was found upon slides from some of the monkeys studied (Table 3). The trophozoites present possessed the typical appearance of *Endamoeba histolytica* from man. The two large lobular pseudopodia shown in Fig. 5 are suggestive of active movement. Food bodies of various sorts were included in the organism. The rounded nucleus appeared to be more delicate than cyst nuclei. Precystic stages (Fig. 1) were highly vacuolated, and possessed a small nucleus, showing only peripheral chromatin, a central karyosome, and several radii.



The chromatoids in the cysts were very much like those in cysts of *E. histolytica* from man. Although variations occurred, they characteristically were large, usually regular in outline, bar-like or fusiform in shape, and few in number. The glycogen vacuole, although infrequent, was clear and usually large.

Table I shows that the percentages in regard to nuclear number of *E. histolytica* cysts from monkey material compare well with those of

TABLE 1.—Nuclear number in cysts of *E. histolytica* from monkey and man

No. of nuclei	100 cysts from			
	Monkey	Homo No. 1	Homo No. 2	Homo No. 3
1-nucleate .....	15	19	7	44
2-nucleate .....	25	24	20	12
3-nucleate .....	4	2	4	3
4-nucleate .....	56	55	66	41
8-nucleate .....	..	..	3	..

cysts of the species from human hosts. Even with a large number of trophozoites present, thus reducing the possibility of mature cysts, the number of quadrinucleate cysts from human host No. 3 is still close to half the sum of tabulated cysts.

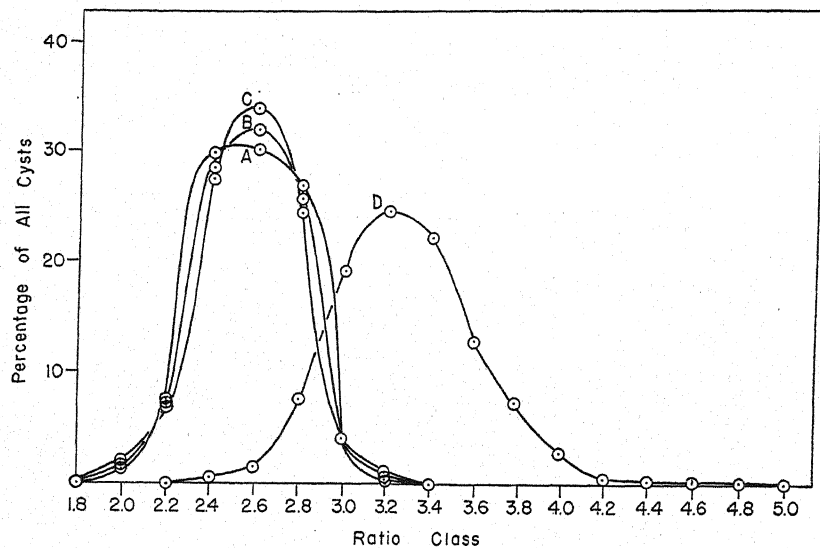
A study was made of the size relationship between the uninucleate cyst and its nucleus, expressed as the ratio of the mean diameter of the cyst to the mean diameter of the contained nucleus. Cysts possessing nuclei in mitotic stages were excluded because of their disturbing effect upon the general cyst-nucleus ratio. The results of this mensurative analysis, and their comparison with those obtained, in the same way, from uninucleate cysts of *E. histolytica* from man are shown in Table 2 and Graph I.

If the value of  $t$  is less than 2, then the difference between the means of different samples is not likely to be significant. Likewise, if the value of  $t$  exceeds 2, the difference is probably significant. Davenport and Ekas (1936) state that, "Of course, the more the difference between means exceeds the limit of  $2 \times$  the standard error of the means, the more certainly significant the difference becomes." Since  $t$  in Table 2 is less than 1, the difference in the means of the samples from man and from monkey is certainly not significant.

Two size races of the *E. histolytica* organisms in the monkey have been determined. In the measurements, only uninucleate cysts were involved. From one host, a race with a mean of  $7.9 \mu$  was discerned with the following range:

Diam. in $\mu$ .....	6.0	7.0	8.0	9.0	10.0
No. of cysts .....	1	4	22	7	1 = 35

This size race is comparable in size and appearance to the small race of *E. histolytica* in man, commonly called *E. hartmanni*.



GRAPH I. Cyst-nucleus ratios: A—*E. histolytica* (man); B—*E. histolytica* (man and monkey); C—*E. histolytica* (monkey); D—*E. chattoni*.

The other size race possessed a mean of  $11.28\mu$  and the following range:

Diam. in $\mu$ .....	8.0	9.0	10.0	11.0	12.0	13.0	14.0
No. of cysts .....	1	12	25	68	54	17	1=178

The cytoplasmic parasite, *Sphaerita* sp., has been observed in one cyst (Fig. 10).

This species was of small numbers in the material from the monkey. Since the number of hosts and the amount of material have been quite extensive, this condition suggests that this species of *Endamoeba* is not of extensive occurrence in the natural state of monkeys in general.

TABLE 2.—Cyst-nucleus ratios of *E. histolytica* in man and monkey

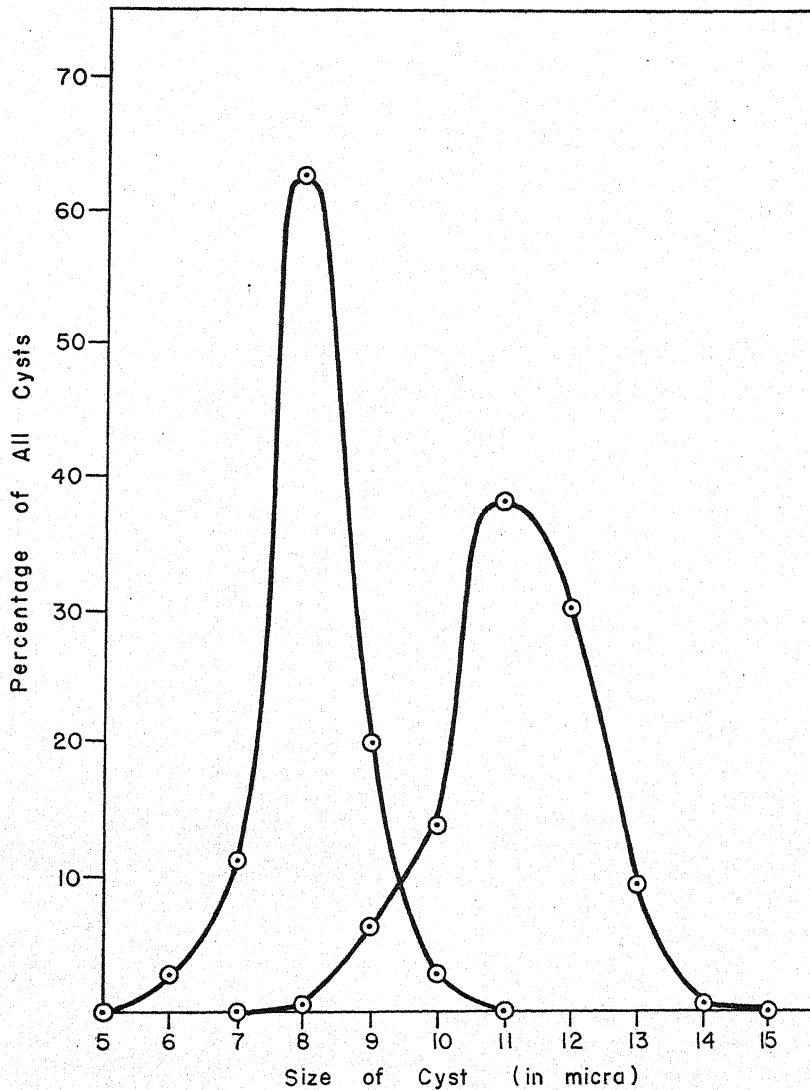
Ratio class	No. of cysts in class		
	Man	Monkey	Combined series
1.90-2.09	3	4	7
2.10-2.29	19	14	33
2.30-2.49	75	55	130
2.50-2.69	77	68	145
2.70-2.89	68	49	117
2.90-3.09	10	8	18
3.10-3.29	1	2	3
N =	253	200	453
*Mean =	2.57	2.59	2.58
* $\sigma$ =	0.198	0.215	0.210
S. E. =	$\pm 0.012$	$\pm 0.015$	$\pm 0.010$
t =	$0.964 = \frac{\text{difference between means}}{\text{standard error of difference between means}} = \frac{\text{Mean}_B - \text{Mean}_A}{\sqrt{S. E.^2_B + S. E.^2_A}}$		

\* The method used in calculating the mean and its standard deviation was the "short-cut" procedure explained in Hegner and Andrews (1927).

*Endamoeba chattoni* Swellengrebel

This uninucleate species is much more characteristic of the monkey than is *Endamoeba histolytica*. *E. chattoni* not only was found in more hosts (Table 3), but was also far more numerous wherever it was disclosed. If the material utilized here is representative of monkeys in general, then these uninucleate cysts are to be considered as typical of monkeys in general.

Representative trophozoites (Fig. 11), of the few encountered, dis-



GRAPH II. Size races of *E. histolytica* (in monkey).

played a somewhat vacuolated endoplasm containing food bodies, apparently bacteria. Where ectoplasm was to be distinguished, the pseudopodia were broad, and only slightly extended. The nucleus was of the *E. histolytica* type, and presented a regular round or oval shape.

The uninucleate nature of *Endamoeba chattoni* was reaffirmed. No cysts above a binucleate stage were observed. Of 850 cysts examined in a study, only 8 binucleate cysts were seen. Thus, the uninucleate cysts definitely constitute the mature stage of development, and the binucleate cysts compose a supernucleate stage of an average incidence of less than one per cent. The binucleate cysts are commonly larger than the uninucleate, just as supernucleate cysts are usually larger among other endamoebae.

The size relationships between the cyst and the nucleus in the uninucleate cysts have been studied in the same way as those of *E. histolytica*. The data are presented in Table 4 and Graph I. The mean of the cyst-nucleus ratios of the *E. histolytica* cysts from man, the mean of those of the same species in the monkey, and the mean of the combined

TABLE 3.—Incidence of *E. histolytica*-like amoebae in monkeys studied

Monkey host	Species of <i>Endamoeba</i>		Source of host
	<i>histolytica</i>	<i>chattoni</i>	
Anubis baboon ( <i>Papio anubis</i> )	—	+	Phila. Zool. Garden
Gelada baboon ( <i>Theropithecus gelada</i> )	+	+	" " "
Guinea baboon (young) ( <i>Papio papio</i> )	+	—	W. Buck's Menagerie
Mandrill (young) ( <i>P. sphinx</i> )	—	+	" " "
Mandrill ( <i>P. sphinx</i> )	+	+	Phila. Zool. Garden
Dwarf monkey ( <i>Cercopithecus talapoin</i> )	+	+	" " "
Green monkey ( <i>C. callitrichus</i> )	+	+	" " "
Japanese macaque ( <i>Silenus fuscatus</i> )	+	—	" " "
Kra monkey ( <i>S. trus</i> )	—	+	" " "
Kra monkey ( <i>S. trus</i> )	—	+	" " "
Mangaby monkey ( <i>Cercopithecus</i> sp.)	—	+	" " "
White-crowned mangaby monkey ( <i>C. aethiops</i> )	+	+	" " "
Mona monkey ( <i>Cercopithecus mona</i> )	+	—	W. Buck's Menagerie
Mona monkey ( <i>C. mona</i> )	+	+	" " "
Mona monkey ( <i>C. mona</i> )	—	+	" " "
White-nosed monkey ( <i>C. fantiensis</i> )	—	+	Phila. Zool. Garden
Rhesus monkey ( <i>Silenus rhesus</i> )	+	+	" " "

ratios of these two groups is in each case of very great significance, since the values of *t* are far above 2.

Although the nucleus is of the *E. histolytica* type, there is an apparent distinction in that the karyosome is smaller, the chromatin granules between the karyosome and the peripheral chromatic granules are finer, and the perikaryosomal ring of granules is less constricted about the karyosome. The peripheral chromatin also appears less dense. The nucleus is thus of more delicate nature, just as that of *E. histolytica* is more delicate than that of *E. coli*. The amount of chromatin, especially karyosomal, is, therefore, in direct proportion to the number of nuclear divisions to be undergone in the cyst.

TABLE 4.—Cyst-nucleus ratios of *E. chattoni*

Ratio class	No. of cysts in class
2.30–2.49	3
2.50–2.69	8
2.70–2.89	43
2.90–3.09	107
3.10–3.29	138
3.30–3.49	123
3.50–3.69	72
3.70–3.89	41
3.90–4.09	16
4.10–4.29	3
4.30–4.49	1
4.50–4.69	1
4.70–4.89	1
N =	557 + 453 = 1010 = Total ( <i>E. chattoni</i> + <i>E. histolytica</i> (man and monkey))
Mean = 3.29	
$\sigma = 0.327$	
S. E. = $\pm 0.014$	
<i>E. chattoni</i> vs. <i>E. histolytica</i> (man), $t = 38.7$	
<i>E. chattoni</i> vs. <i>E. histolytica</i> (monkey), $t = 33.4$	
<i>E. chattoni</i> vs. <i>E. histolytica</i> (man and monkey), $t = 41.8$	

Large numbers, small size, and irregularity in shape characterize the chromatoids in the cysts. The usual chromatoids are small and rounded or irregularly angular (Fig. 15). At times, small granular chromatoids, as many as 250 in a single cyst, would obscure or hide the nucleus. To estimate the number in cysts containing masses of minute particles is almost impossible. The lower numerical limits are about 15–20 chromatoids, and the average number may be set conservatively at 40–60 chromatoids. The larger cysts do not appear to contain more chromatoids despite a maintained smallness in chromatoid size (Fig. 13).

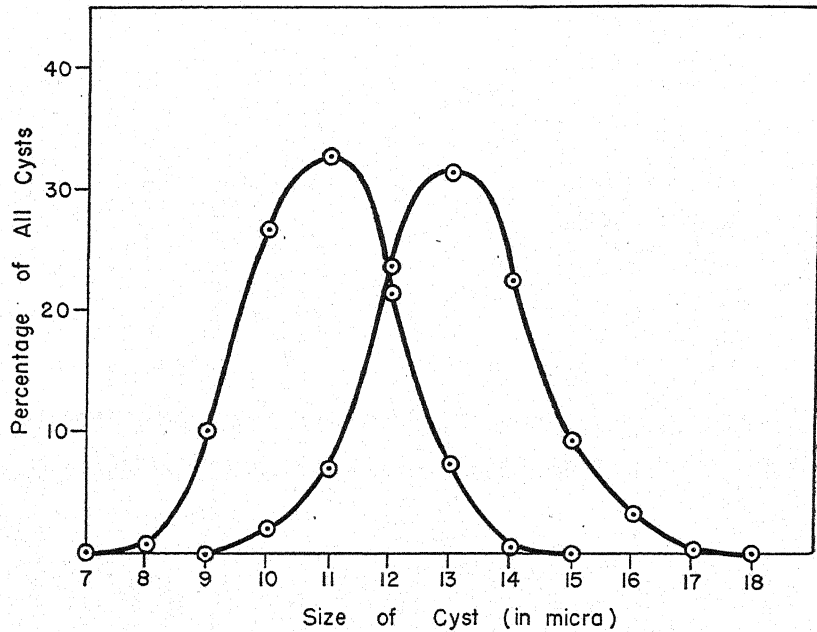
A strain of *E. chattoni*, seen in three hosts, presented less numerous chromatoids, some of which were large in size and regular in shape. Most frequent were cysts containing 15–20 chromatoids of highly diversified shape and size. A series of chromatoidal conditions could be distinguished in which the size decreased and the shape became more irregular with increase in number. This began with a cyst containing two large and regular bar-shaped chromatoids (Fig. 17), and concluded with one having approximately sixty-two chromatoids of characteristic types (Fig. 18). Paired chromatoids of the more regularly contoured kind were frequent throughout the series.

Commonly found within the cysts was a small, dense, homogeneously stained area, of a light greyish brown color. This round or oval area has been interpreted as a glycogen vacuole, possibly a remnant. It is small in comparison with that found in *E. histolytica*.

Vacuoles of puzzling nature were found in a few cysts, usually occupying roughly half the cyst diameter. The vacuole, containing the nucleus, would be outlined generally by the small chromatoids lying around it, a characteristic of the glycogen vacuole in the binucleate cystic stages of *Endamoeba coli*. Fig. 14, however, shows chromatoids both



inside and outside the vacuole, whose boundary is clearly seen. The area within the vacuole was clearer than the circumjacent cytoplasm, suggesting a former glycogen content later dissolved out. But, despite the similarity to the binucleate stage in *E. coli*, the location of the nucleus and even chromatoids within the vacuole renders this consideration improbable.



GRAPH III. Size races of *E. chattoni*.

Size races were not easily established, but two races were measured. The smaller race was obtained from four hosts and furnished the following data:

Diam. in $\mu$ .....	8.0	9.0	10.0	11.0	12.0	13.0	14.0
No. of cysts .....	3	38	104	127	83	29	2=386
Mean = 10.93 $\mu$							
$\sigma = 1.051$							
S. E. = $\pm 0.053$							

The larger race, from three hosts, was recognized by the following measurements and data:

Diam. in $\mu$ .....	10.0	11.0	12.0	13.0	14.0	15.0	16.0	17.0
No. of cysts .....	8	28	95	127	90	38	13	1=400
Mean = 13.06 $\mu$								
$\sigma = 1.178$								
S. E. = $\pm 0.059$								

The means of these two size races are of a definitely significant difference, since in their comparison the value of *t* is 26.7, far above 2.

## DISCUSSION

Observation of stained preparations of the so-called *E. histolytica*-like amoebae in monkeys has established by the comparison in the cysts of chromatoids, glycogen areas, nuclear number in developmental stages, cyst-nucleus size relationship, and nuclear structures, that there can be differentiated two species of amoebae. This is also somewhat corroborated by the restricted study of the trophozoite stages.

Since the more recent literature largely expresses the opinion that the *E. histolytica*-like amoebae in monkeys are very similar to, if not identical with *Entamoeba histolytica* in man, an important result of the studies reported in this paper is the definite identification of *E. chattoni*.

Chatton (1912) was observing cysts of *E. chattoni* among his "trophozoites" of "*Löschia* sp." from *Macacus sinicus*. Not only are there no polynucleates, but the measurement of the cysts and nuclei in his five drawn uninucleate "trophozoites" disclosed a mean ratio of 3.3 and a range of 3.1 to 3.6.

Swellengrebel (1914) observed in *Macacus rhesus* the organisms described by Chatton, and believed them to be a new species, *Entamoeba chattoni*. Aside from a few binucleate cysts, no polynucleate cysts were found. He described the trophozoites as alveolar, mostly free of inclusions, and with very little ectoplasm. Of five drawn uninucleate cysts, the mean cyst-nucleus ratio was found to be 3.2, with a range of 2.9 to 3.6.

Behrend (1914), aside from his seeming confusion of *E. histolytica*- and *E. coli*-like cysts, appears to have seen both *E. histolytica* and *E. chattoni* in material from *Macacus rhesus*. The former species is indicated by the description of quadrinucleate cysts, often with single large chromatoids. But uninucleate cysts with paired thin chromatoids, and the rarity of polynucleate cysts indicate the probable presence of *E. chattoni* cysts.

In this period, Castellani (1908) had erected the first new species, *Entamoeba nuttali*, based upon trophozoites of vague morphology from *Macacus pileatus*, and Mathis (1913) had proposed the new species, *Löschia duboscqi*, apparently for organisms of *Entamoeba histolytica* from *Macacus rhesus* and *M. tchliensis*.

Bach (1923) and Mello (1928) studied similar trophozoites and cysts from *Macacus rhesus*. Bach presents five drawings of uninucleate cysts whose cyst and nucleus measurements furnished a mean ratio of 2.6, with a range of 2.4 to 2.9. Since groups of ten cysts were found sufficient to approximate the mean ratio, there is no doubt that Bach was considering cysts of *E. histolytica*. There remains the possibility of his ignoring *E. chattoni*, since he mentions occasional cysts filled with "splintered and short rod-shaped chromotoids," and discloses that most

of the cysts were uninucleate. In addition, most of the trophozoites possess small, bluntly lobular pseudopodia, and are 13–15  $\mu$  in size. These resemble the one depicted in Fig. 11. Thus Bach probably confused the two species and drew cysts of only one.

Hegner and Chu (1930) definitely were studying cysts of *E. chattoni* from wild monkeys, *Macacus philippinensis*. They refer to their necessity to rely on uninucleate cysts, which "contained many paired chromatoidal bodies of various sizes." No polynucleate cysts are mentioned, but a binucleate is drawn together with a uninucleate cyst, whose cyst-nucleus ratio is 3.32. The trophozoite shown is similar to the one (Fig. 11) in this paper. From mean cyst and nucleus diameters of 100 cysts a cyst-nucleus ratio of 2.91 is found. What factors bring the ratio below 3.3 cannot be gleaned from the report.

On the other hand, amoebae maturing with quadrinucleate cysts and morphologically the same as *E. histolytica* from man have been found in *Macacus rhesus* and *M. sinicus* by Dobell and Laidlow (1926) and Dobell and Bishop (1929), to which hosts Dobell (1928, 1931) also added *M. nemestrinus*; in a chimpanzee by Hegner and Schumaker (1928); and in *M. irus*, *M. rhesus*, *M. sancti-johannis*, and *M. lasiotis* by Kessel (1928). A more extensive study by Mackinnon and Dibb (1938) established as hosts of these amoebae "a Gorilla, four Chimpanzees, two Gibbons (*Hylobates lar* and *H. lar lenciscus*), a Guinea Baboon (*Papio papio*), a White-crowned Mangaby (*Cercocebus aethiops*), a Sooty Mangaby (*C. fuliginosus*), a Mona Monkey (*Cercopithecus mona*), a Schmidts' Monkey (*C. ascanius schmidtii*) and a Patas Monkey (*Erythrocebus patas*)."

The organisms of *E. chattoni* encountered in the literature belong, apparently, almost entirely to the strain described in this paper as producing less numerous and more regularly shaped chromatoids. Drawings depict no more than 25 chromatoids, most of which are of regular shape.

The definite establishment of the uninucleate species in the monkey adds another to the list of such species with *Endamoeba* nuclei. These species have been found in domestic cattle, the horse, the Chinese sheep, the goat, the gnu and in swine.

A brief study of cysts of a species with uninucleate cysts, believed to be *Endamoeba bovis*, in cattle has been made by the author from slides furnished by Dr. Wenrich. Measurement of cyst and nuclear diameters in 20 cysts provided a ratio of 3.22. Chromatoids were typically few in number, but were irregular in shape and of varying sizes. The glycogen vacuoles were clear. The fundamental cyst-nucleus ratio and the irregularity of the chromatoids definitely link this species to that in the monkey. A like similarity of the other species remains to be investigated.

The reports of amoebic dysentery in monkeys naturally raises the question as to which species is the causative agent. From the descriptions, amoebae of the *Endamoeba histolytica* type were responsible. This would be analogous to the situation in man, where *E. histolytica* is considered a primary factor, if not the causative agent, in amoebic dysentery. In addition, the low incidence of this disease in the monkey, coupled with the heavy amoebic infections found among these animals, may be explained in terms of the scarceness of *E. histolytica* in relation to *E. chattoni* in these hosts. By analogy with the other uninucleate species mentioned, which do not appear to be detrimental to their various hosts, it may be expected that the non-pathogenicity of *E. chattoni* will be confirmed.

Research has already been carried out upon the pathogenic role of *Endamoeba histolytica* in the monkey. Among others, Kessel (1928) and Dobell (1931) have performed transfers of amoebae of this type from monkeys to kittens, in which amoebic dysentery has been produced by the organisms. The clinical and pathological evidence from both monkeys and kittens also supports the identity of these amoebae with *E. histolytica* in man. Because of this, the effects of chemical treatment on the pathogenic organism in the monkey have been investigated. Emetine, as in man, has been found to be effective by Dobell and Bishop (1929), who state, "it is suggested that macaques can therefore be utilized . . . in place of men in future chemotherapeutic experiments directed towards the discovery of remedies for human amoebic dysentery." Such experiments, however, must be performed only on monkeys parasitized by *E. histolytica*. The confusion of *E. chattoni* with the dysentery amoeba, as may have occurred in the past, would render invalid the results of such experimentation.

#### SUMMARY

1. The morphology of the *E. histolytica*-like amoebae in monkeys has been studied in prepared material from twelve different species of monkeys, including seventeen individual hosts.

2. By the use of several differential methods, both *Endamoeba histolytica* and *Endamoeba chattoni* have been identified.

3. The cysts of *E. histolytica* present a cyst-nucleus diameter ratio of 2.6, and two size races with means of  $7.9\mu$  and  $11.3\mu$  were recognized. The organisms are identical with those of *E. histolytica* in man.

4. *E. chattoni* Swellengrebel is validated. It is uninucleate, with a binucleate supernuclear stage of less than one per cent incidence, has a cyst-nucleus diameter ratio of 3.3, and includes at least two size races with means of approximately  $10.9\mu$  and  $13.1\mu$ . Two strains are discernible through their characteristic chromatoids.

5. These uninucleate organisms are believed to be related closely to uninucleate species of similar nature that have been recognized in other animals.

6. Available evidence indicates that *E. chattoni* is non-pathogenic in the monkey.

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## EXPLANATION OF PLATES

## PLATE I

All figures are of organisms fixed with Schaudinn's fluid, containing 5% of glacial acetic acid, and stained by the iron-haematoxylin method. Figs. 2, 3, 4 are from the same host, and likewise for Figs. 6, 7, 9, 10. Dimensions are expressed in mean diameters. Magnification 2800  $\times$ .

*Endamoeba histolytica*  
(in monkey)

FIG. 1. Precystic form of smaller race in a young guinea baboon (*Papio papio*). Size 7.05  $\mu$ .

FIG. 2. Average-sized uninucleate cyst of smaller race in a mona monkey (*Cercopithecus mona*). Size 8.0  $\mu$ . Nuclear size 3.3  $\mu$ . Cyst-nucleus ratio 2.42.

FIG. 3. Binucleate cyst of smaller race in a mona monkey. Remains of a large chromatoid. Size 8.5  $\mu$ .

FIG. 4. Quadrinucleate cyst of smaller race in a mona monkey. Size 8.0  $\mu$ .

FIG. 5. Elongate trophozoite in a white-crowned mangaby monkey (*Cercocebus aethiops*). Size 23.5  $\mu \times 11.3 \mu$ .

FIG. 6. Average-sized uninucleate cyst of larger race in a mona monkey. Size 10.85  $\mu$ . Nuclear size 4.25  $\mu$ . Cyst-nucleus ratio 2.55.

FIG. 7. Binucleate cyst of larger race in a mona monkey. Size 12.2  $\mu$ .

FIG. 8. Trinucleate cyst of larger race in a gelada baboon (*Theropithecus gelada*). Size 10.35  $\mu$ .

FIG. 9. Quadrinucleate cyst of larger race in a mona monkey. Size 12.2  $\mu$ .

FIG. 10. Uninucleate cyst of larger race in a mona monkey. Parasitized by *Sphaerita* sp. Size 12.2  $\mu$ . Nuclear size 5.65  $\mu$ . Cyst-nucleus ratio 2.16.

## PLATE II

All figures are of organisms prepared as in Plate I. Figs. 13, 15 are from the same host, likewise for Figs. 12, 16, and also for Figs. 17, 18, 19. Dimensions are expressed in mean diameters. Magnification 2800  $\times$ .

*Endamoeba chattoni*

FIG. 11. Trophozoite stage in a mangaby monkey (*Cercocebus* sp.). Size 16  $\mu$ .

FIG. 12. Average-sized uninucleate cyst of smaller race in an anubis baboon (*Papio anubis*). Small glycogen vacuole remnant. Size 11.3  $\mu$ . Nuclear size 3.8  $\mu$ . Cyst-nucleus ratio 2.97.

FIG. 13. Average-sized uninucleate cyst of larger race in a kra monkey (*Silenus irus*). Size 13.2  $\mu$ . Nuclear size 3.8  $\mu$ . Cyst-nucleus ratio 3.47.

FIG. 14. Uninucleate cyst of larger race in a kra monkey. Large vacuole with definite boundary, containing nucleus and some chromatoids. Size 14.6  $\mu$ . Nuclear size 4.25  $\mu$ . Cyst-nucleus ratio 3.43.

FIG. 15. Uninucleate cyst of smaller race in a mona monkey (*Cercopithecus mona*). Large number of small chromatoids. Size 12.2  $\mu$ . Nuclear size 3.8  $\mu$ . Cyst-nucleus ratio 3.21.

FIG. 16. Binucleate stage in an anubis baboon. Size 16.45  $\mu$ .

Figs. 17-19 represent the strain with fewer, more regular chromatoids.

FIG. 17. Uninucleate cyst in a gelada baboon (*Theropithecus gelada*). Two massive and regular chromatoids, and remnant of glycogen vacuole. Size 12.2  $\mu$ . Nuclear size 3.8  $\mu$ . Cyst-nucleus ratio 3.21.

FIG. 18. Uninucleate cyst in a gelada baboon. Many varying chromatoids. Size 12.7  $\mu$ . Nuclear size 3.8  $\mu$ . Cyst-nucleus ratio 3.37.

FIG. 19. Binucleate cyst in a gelada baboon. Chromatoids of more typical number and appearance. Size 16.9  $\mu$ .

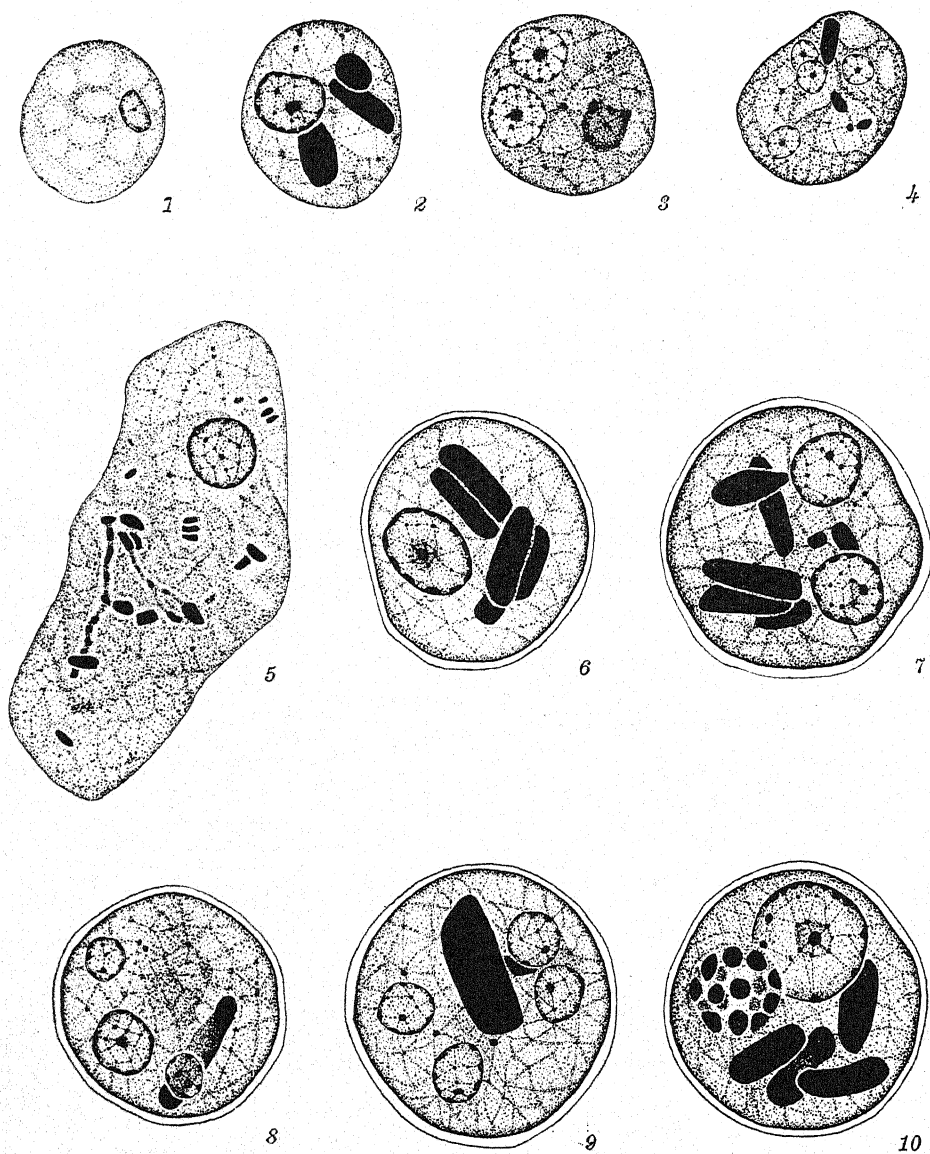


PLATE I

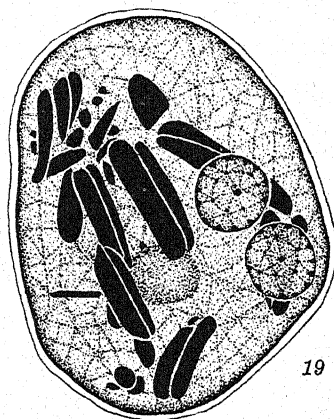
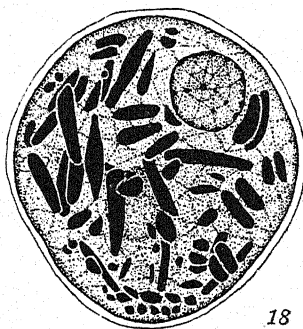
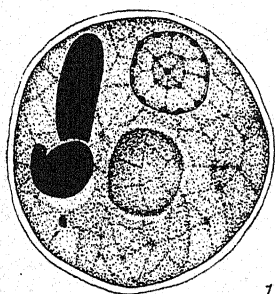
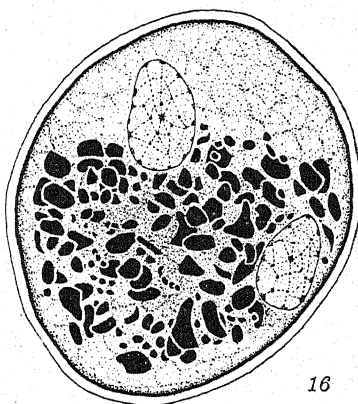
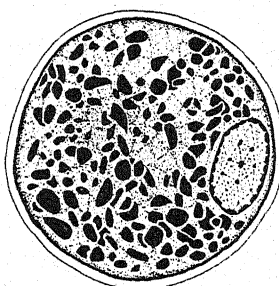
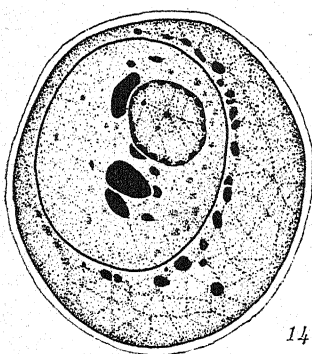
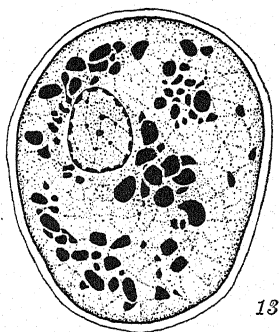
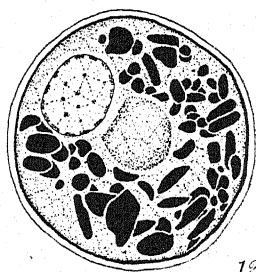
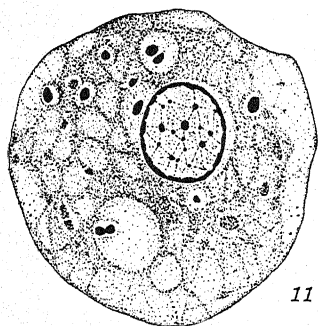


PLATE II





CERCARIA DOUGLASI CORT, 1917 AND ITS RELATION  
TO THE CERCARIA OF *COTYLURUS*  
*FLABELLIFORMIS* (FAUST, 1917)\*

LOUIS OLIVIER AND W. W. CORT

*Cercaria douglasi* was described briefly by Cort (1917) from a species of *Physa* from the Douglas Lake Region, Michigan. Later Cort and Brooks (1928) identified as this species cercariae from *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla* Walker, *Stagnicola emarginata angulata* (Sowerby), and *Physa parkeri* Currier. Their description, however, was from the cercariae that emerged from three specimens of the varieties of *L. stagnalis*.

Subsequent investigations have revealed that cercariae in the Douglas Lake Region, previously identified as *C. douglasi*, actually represent two distinct but closely related species. One of these, which is found only in members of the PHYSIDAE, we identify as *C. douglasi* Cort, 1917; the other, which develops only in members of the LYMNAEIDAE, was mistakenly identified by Cort and Brooks as *C. douglasi*. This second species has been shown by van Haitsma (1930) to be the cercaria of *Cotylurus flabelliformis* (Faust, 1917).

Since Cort's original description of *C. douglasi* was very brief and was made from cercariae obtained by autopsy from a single snail, a redescription of this species is presented. Also, *C. douglasi* is compared with the cercaria of *C. flabelliformis* with which it has been confused.

REDESCRIPTION

*Cercaria douglasi* Cort, 1917  
(Figs. 1-5)

*Specific diagnosis:* Strigeid cercaria. (Measurements in Table 1). Oral opening terminal, prepharynx short, pharynx small, esophagus bifurcating about half way between pharynx and level of ventral sucker. Ceca long and narrow, inconspicuous, terminating at level of genital primordium anterior to excretory bladder. Four small, inconspicuous penetration glands anterior to ventral sucker and ventral to ceca. Narrow gland ducts run anteriorly, penetrate oral sucker, in which they are only slightly dilated, and open terminally lateral to oral opening. Excretory system typical. Two pairs of flame cells on each anterior collecting tubule; three pairs on each posterior collecting tubule, two pairs in body and one pair near base of tail stem. A transverse commissure immediately anterior to level of ventral sucker connects anterior portions of bladder arms. A band of 6-8 irregular rows of fine, closely set spines about anterior portion of oral sucker. Ventrally, scattered spines as far back as the anterior margin of genital primordium in lateral fields

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\* Contribution from the University of Michigan Biological Station, the Biological Laboratory, New York University, University Heights, and the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.



and as far as ventral sucker centrally. Dorsally, scattered spines just beyond level of pharynx centrally and slightly farther back in lateral fields. About 16–20 long, thin “forward projecting” spines dorsal to oral opening (Fig. 3). Ventral sucker bears several irregular rows of thin, elongate spines. A pair of hair-like processes laterally at level of genital primordium. Hair-like processes on tail stem. Inconspicuous stellate cells grouped along excretory tubule of tail stem. “Island of Cort” small. A pair of clear areas (“unpigmented eyespots”) laterally anterior to ventral sucker. Second intermediate hosts are members of Physidae in which tetracotyle larvae very similar to those of *C. flabelliformis* are formed.

*Hosts:* *Physa parkeri* Currier from Douglas Lake, Cheboygan County, Michigan. *Physa gyrina* Say from Grace Harbor, Presque Isle County and Munro Lake, Cheboygan County, Michigan.

Like the cercaria of *Cotylurus flabelliformis*, *C. douglasi* emerges from the snail host during the daytime. The cercariae swim about constantly with tail foremost and frequently change direction. Since they tend to revolve on the long axis as they swim, they often take an irregular spiral course. They tend to be evenly distributed throughout the water in a small container and differ in this respect from the cercariae of *C. flabelliformis* since the latter tend to collect near the surface. This description of the activity of the cercaria of *C. flabelliformis* differs from that given by Cort and Brooks (1928, pp. 191–192); it seems possible that their description was made from old cercariae.

*Cercaria douglasi* appears to be smaller in all dimensions than the cercaria of *C. flabelliformis* (Table 1). The most striking and clear-cut size difference is in the length of the tail stem, which in *C. douglasi* is always shorter than the body while in the cercaria of *C. flabelliformis* it is always longer. This difference makes it possible to differentiate the two species readily even under low magnification.

TABLE 1.—Measurements of the cercaria of *Cotylurus flabelliformis* and *Cercaria douglasi*. Both lots of cercariae were fixed in hot 5% formalin by the same method and measurements of 50 of each species made by the same person. All measurements are in microns. The mean with its probable error is given and the extremes are included in parenthesis.

	Cercaria of <i>Cotylurus flabelliformis</i> from the varieties of <i>Lymnaea stagnalis</i>	<i>Cercaria douglasi</i> from <i>Physa gyrina</i>
Body length .....	217 ± 3 (185–250)	198 ± 2 (172–224)
Body width .....	39 ± 0.5 (32–51)	38 ± 0.3 (32–45)
Tail stem length .....	238 ± 1 (211–256)	184 ± 0.5 (173–192)
Tail stem width .....	36 ± 0.4 (32–45)	32 ± 0.3 (31–38)
Furcal length .....	239 ± 1 (211–262)	207 ± 1 (186–218)
Length of oral sucker .....	40 ± 0.3 (32–47)	35 ± 0.4 (29–43)
Width of oral sucker .....	20 ± 0.1 (18–23)	18 ± 0.1 (13–20)
Diameter of ventral sucker ....	24 ± 0.1 (21–26)	23 ± 0.1 (21–26)

The two species may also be differentiated on the basis of body spination (Figs. 4 and 5). On the dorsal surface the pattern of spination is virtually the same in both species, but ventrally the spines on the cercaria of *C. flabelliformis* extend to the posterior limits of the body, while on *C. douglasi* they extend only to the level of the genital primordium. In addition, the spines on the cercaria of *C. flabelliformis* are larger and more conspicuous than those of *C. douglasi* so that while the posterior limits of the spination of the latter were difficult to determine, it was relatively easy to determine the extent of spination on the former.

Finally, there appear to be clear-cut differences between *C. douglasi* and the cercaria of *C. flabelliformis* in their choice of second as well as first intermediate hosts, the metacercariae in each case normally developing in snails of the same family as the snails used as first intermediate hosts, i.e., PHYSIDAE for *C. douglasi* and LYMNAEIDAE for *Cotylurus flabelliformis*.

#### SUMMARY

A redescription of *Cercaria douglasi* Cort, 1917, is presented. This species has been confused with the cercaria of *Cotylurus flabelliformis* (Faust) (= *C. douglasi* Cort and Brooks, 1928). The cercariae of these two strigeid species may be distinguished readily by differences in body spination and relative lengths of their tail stems. *Cercaria douglasi* develops only in members of the PHYSIDAE and members of this family are also the normal second intermediate hosts. The cercaria of *C. flabelliformis* develops only in lymnaeid snails and certain of the LYMNAEIDAE are its normal second intermediate hosts.

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#### EXPLANATION OF PLATE, p. 346

FIG. 1. Sketch of *C. douglasi* drawn from a living specimen under slight cover glass pressure to show proportions.

FIG. 2. Body and portion of tail of *C. douglasi*. Ventral aspect.

FIG. 3. En face view of *C. douglasi* showing oral opening, gland duct openings and forward projecting spines.

FIG. 4. Body of *C. douglasi*. Ventral aspect. The spined area is indicated by shading.

FIG. 5. Body of the cercaria of *Cotylurus flabelliformis*. Ventral aspect. The spined area is indicated by shading.

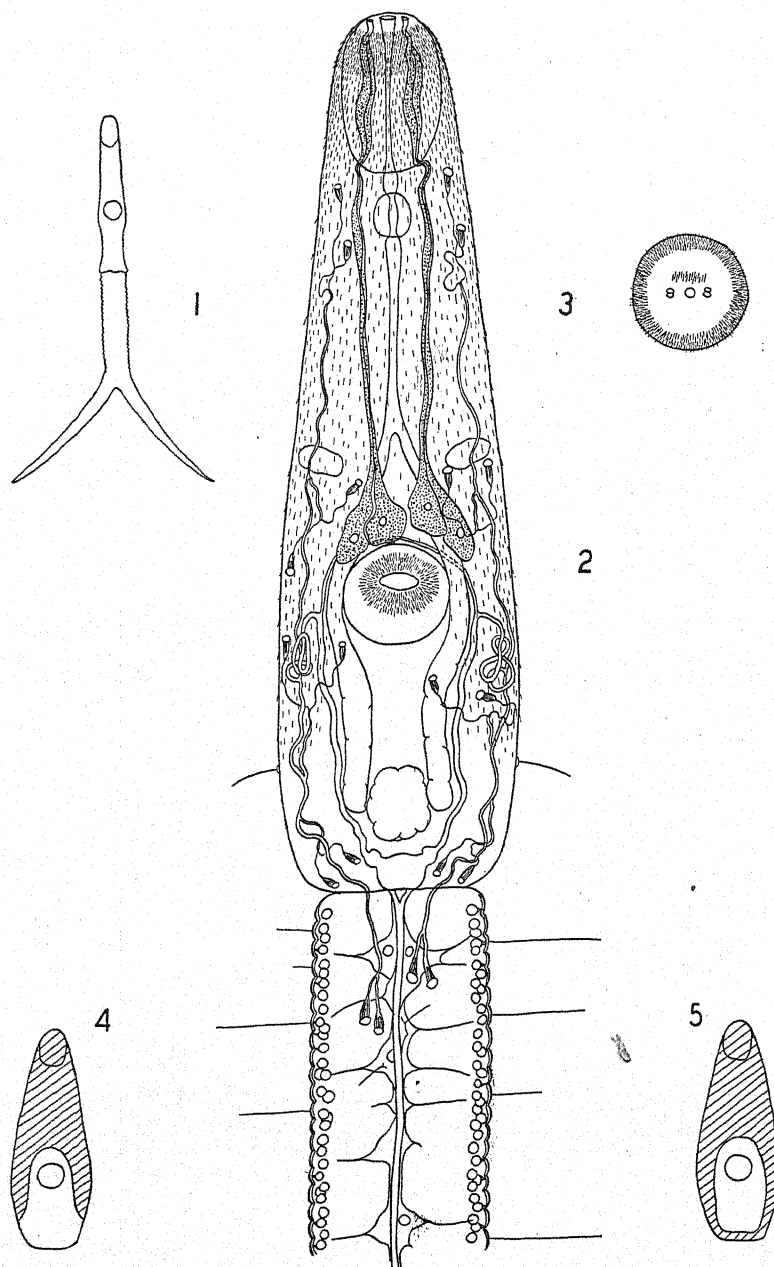


PLATE I

THE LIFE HISTORY OF *ECHINOCHASMUS DONALDSONI*  
N. SP., A TREMATODE (ECHINOSTOMIDAE)  
FROM THE PIED-BILLED GREBE\*

PAUL C. BEAVER  
Lawrence College

In August, 1939, about one-half per cent of *Ammicola limosa* and *A. lustrica* taken from Hook Point on Douglas Lake and from a pond in Wilderness Park, Michigan, were infected with a minute undescribed species of echinostome-like cercaria. The first collections were made by Mr. Alan Donaldson who was surveying cercarial infections in the small operculate snails, and who kindly gave me the echinostome material. Additional material was obtained by numerous subsequent collections. After the cercaria and redia were described, it was possible to undertake a limited number of experiments in an attempt to discover other segments of the life cycle. It was found that freshwater fish are second intermediate hosts and the adult is an undescribed species of *Echinochasmus* which matures in the duodenum of pigeons (experimental) and the pied-billed grebe (*Podilymbus podiceps*). The name *Echinochasmus donaldsoni* is proposed for the new species.

THE CERCARIA

(Fig. 7)

The cercariae emerge in the early morning and are active for 36 to 48 hours in the laboratory. They are extremely small. Swimming behavior is somewhat like that of many stylet cercariae. They swim echinostome-like upwards for a few seconds, then fall slowly with the body flexed ventrally and with the tail contracted and held upward over the ventral side of the body. Creeping is infrequent. Hanging from the surface film is common in water less than a centimeter deep. The body of falling cercariae is circular in outline when seen from above. The tail is long and slender when swimming but may be contracted to less than one-half the length of the extended body when creeping.

*Description:* Body spindle-shaped except when extended, widest at middle; length 70 to 120  $\mu$  in various states of contraction. Tail length about equal that of body. Oral sucker slightly ventral, spherical; diameter slightly greater than that of acetabulum. Acetabulum in posterior third of body, bearing 26 small scale-like

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\* A contribution from the University of Michigan Biological Station and the Department of Biology, Lawrence College, Appleton, Wisconsin.

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spines on its margin. Oral sucker bears 10 minute spines on inner dorsal margin and 5 on either side of the outer ventral margin. Collar and collar spines not discernible. Cuticular spines of body undeveloped. Prepharynx relatively long; pharynx cylindrical to spindle-shaped, not pyriform; length less than one-half diameter of oral sucker. Esophagus and ceca plain with open lumina; usually obscure. Cystogenous glands composed of angular masses of fine rods, mostly confined to a lateral field on either side extending from level of pharynx to posterior end, and a median pair of fields between pharynx and acetabulum. Excretory bladder of 2 chambers, both contractile. Excretory siphons arise antero-laterally from anterior chamber of bladder, enlarge immediately and form a single loop between acetabulum and pharynx; siphons narrow rather abruptly at level of pharynx and extend forward to sides of oral sucker. Smaller tubules and flame cells could not be seen. Siphon granules angular and tend to be compound; greatest diameter of largest ones about  $4\mu$ ; 8 to 14 in each siphon, evenly distributed along large part of siphons from excretory bladder to pharynx. Tail powerful; attached slightly ventrad. Sharp circumferential ridges throughout its length is a conspicuous feature in contracted tail of living specimens. Excretory canal extending from bladder into tail forms a large top-shaped bladder which may be emptied by contraction; lateral branches of this caudal excretory structure apparently are absent. Pigment absent from all parts of cercaria. Measurements in microns on fixed specimens: body in moderate state of extension, 100 by 52; tail, 102 by 26; oral sucker, 22 by 20; acetabulum, 21 by 18; pharynx, 10 by 9; distance between suckers, 50; distance between oral sucker and pharynx, 15; contracted specimens, body and tail respectively, 70 by 60 and 50 by 30.

#### THE REDIA

(Figs. 8-10)

*Description:* Living rediae lightly colored with scattered granules of brownish yellow pigment. Collar plain; low but generally visible on both young and old stages. Appendages usually low, sometimes prominent. Birthpore prominent. Pharynx relatively large, its position variable with state of contraction; sometimes withdrawn far into body, bringing collar forward to anterior tip of body where it may form lips of the oral orifice. Gut usually narrow, long, reaching region of appendages in mature rediae and beyond appendages in very young ones. Cercariae in various stages of development in rediae; no mature cercariae were found in rediae and an abundance of larger immature cercariae were observed free in the snails' tissues. Around 3,000 mature and slightly immature cercariae were found in the tissues of one snail. Measurements on living rediae: largest redia, 1.55 by 0.215 mm just back of collar; sucker, 86 by  $77\mu$ ; appendages, 0.34 mm from posterior end. Small rediae, 0.14 mm in length; pharynx,  $30\mu$  wide by  $24\mu$  long. After fixation, collar usually not readily seen; located half-way between birthpore and anterior end. Appendages low; located on posterior one-fifth of body. Gut length as in living state; position ventral, mostly in contact with ventral body wall. Average mature redia, 0.42 by 0.12 mm at level of birthpore; pharynx,  $40\mu$  long by  $44\mu$  wide. Appendages  $80\mu$  from posterior end; collar and birthpore, 50 and  $100\mu$  respectively from anterior end.

#### THE METACERCARIA

(Fig. 6)

Various species of snails, tadpoles, and fish were exposed to cercariae. All experiments with the first 2 were negative, but every attempt to infect fish was successful.

Small fish and active cercariae were placed in shallow aquaria for several hours. Under magnification it could be seen that the respira-



tory currents were carrying the cercariae into the mouth and pharynx, and the cercariae were but rarely reappearing outside the gill chamber. Examination of such test animals invariably revealed small metacercarial cysts on the gills. Parasite free guppies (*Lebistes reticulatus*), perch (*Perca flavescens*), mollies (*Mollienisia latipinna*), and bluegills (*Helio-perca incisor*) were infected in this manner. Additional species which were not definitely known to be parasite free but by exposure and later comparison with control animals from the same lots, were found to be suitable hosts are: mudminnow (*Umbra limi*), bullhead (*Ameiurus nebulosus*), and shiners (*Notropis* spp.). In one of the areas where infected snails were most abundant, minnows were examined for metacercariae which were similar to those found in the experimental minnows. The most abundant species were the five-spined stickleback (*Eucalia inconstans*), fine-scaled dace (*Pfritte neogaeus*), and bowfin (*Amia calva*). Thirty stickle-backs, ranging between 18 and 42 mm in length, carried from 1 to 140 cysts each, with the number of cysts roughly proportional to the size of the fish. Five bowfins ranging between 40 and 50 mm, each carried from 46 to 200 cysts. Twelve dace, around 15 mm in length, carried from 3 to 24 cysts, and 7 larger ones (50 mm) carried from 21 to 170 cysts. In the field hosts the metacercariae were usually in various stages of development.

The mode of infection, encystment, and the nature of development were observed on fish that were infected in the laboratory. Cercariae are carried passively into the gill chamber where they attach themselves to the gill filaments and penetrate the epithelium over or between the lamellae. Occasionally it appears that they establish themselves between the secondary lamellae of smaller fish. In any case, whether their location is deep within the gill tissues or more superficial, they form a relatively strong primary cyst wall immediately. Cysts that are 24 hours old are fairly uniform in size and shape, 68 to 76  $\mu$  long by 40 to 44  $\mu$  wide, by 34 to 36  $\mu$  thick. Where cysts are formed under pressure, the shape may vary slightly. The primary cyst wall is less than 2  $\mu$  thick.

The body of the metacercaria does not differ appreciably from that of the cercaria during the first day after encystment. Collar spines become faintly visible after 3 to 6 days and the cyst wall becomes rough and indefinite on its outer margin due to infiltration cells. At 13 days the body is flexed ventrally, collar spines are small but more clearly defined, the cuticula is beginning to show spination pattern, and the cyst wall including host tissue is 4 to 7  $\mu$  thick. At 21 days the body is flexed almost double, collar spines are about 3  $\mu$  long, cuticular spination is well developed, and the spines around the acetabulum have disappeared. At 4 weeks the spination is slightly advanced and at 5 months the only additional change is in the cyst wall which is 10 to 14  $\mu$  thick and unpig-

mented, and the collar spines which are 5 to 7  $\mu$  long. Accurate measurements were not obtained on both suckers of a single individual but it appeared that the acetabulum was relatively larger after 4 weeks. Size and shape of the cyst wall, number and distribution of siphon granules, and spination of oral sucker remain unchanged after encystment.

#### THE ADULT

(Figs. 1-5)

Metacercariae of various ages were fed to 6 pigeons, 6 canaries, 1 chicken, 1 kitten, 1 rat, and 2 mice. Perch that were taken from a lake where snails do not occur were experimentally infected and after 13 days were fed to 3 canaries and 1 pigeon. All were negative after 8 to 10 days. After 17 to 21 days, perch carrying around 150 metacercariae were fed to a pigeon, and 7 days later 3 worms that were barely mature were recovered from the duodenum. Five-spined stickle-backs were collected from an area where the cercariae were found and the approximate number of metacercariae on their gills was determined. They were then fed to a number of hosts to determine whether they would develop into the same adult as those obtained from the experimental metacercariae. Also it was desirable to determine whether metacercariae, some of which probably would be older than 21 days in the field hosts, would develop in laboratory hosts other than the pigeon. Three canaries were given gills bearing 100 to 300 metacercariae, and were found negative after 6, 7, and 9 days respectively. A rat, 2 mice, and a kitten were likewise fed 100 to 300 cysts and were negative on the 8th day. A month-old chicken was negative on the 8th day after receiving 150 cysts from the sticklebacks. Three pigeons were given 100 to 250 cysts and after 7 and 8 days, 1 and 3 mature worms respectively were recovered from the duodenum of 2 of them. Another pigeon was given over a period of 4 days, 250 sticklebacks, each of which carried 1 to 140 metacercariae. Eggs appeared in the feces on the 7th day following the original feeding, and on the 9th day 31 of the worms were found in the duodenum.

Several conclusions may be drawn from the above described feeding experiments. The metacercariae are infective after 3 weeks of encystment. The pigeon is a suitable laboratory host although the percentage of metacercariae which developed into adults was small, possibly because many of the metacercariae were immature. Maturity in the adult worm is reached on the 7th day in pigeons. Its location in the host is the duodenum. The canary is definitely refractive to this species and although the experiments with the rat, mouse, kitten, and chicken were inconclusive, it seems likely that they too are unsuitable laboratory hosts. The adults obtained from the field infections of metacercariae are the same as those from the experimental infections.

Two pied-billed grebes (*Podilymbus podiceps*) were taken from an area about 25 miles from where the infected snails were collected. In the first one, 25 worms which were identical with those taken from experimental pigeons, were found in the duodenum. Over 100 worms of this species were taken from the second.

The following description is based on sections and toto-preparations of mature specimens from the grebe and pigeon.

*Description:* Mature worms small and elongate, tapered at neck and posterior end. Size, 0.88 by 0.256 mm to 1.84 by 0.32 mm. Widest level usually at, or just posterior to, testes but may be at level of acetabulum, esophagus, or crown. Cuticular spines relatively large, extending to level of posterior testis along lateral and ventral sides, and to ovary on dorsal side. Oral sucker small, terminal, with the mouth directed ventro-anteriorly; provided with a row of 10 scale-like spines on the inner border of the dorsal side and 8 to 10 low papilla-like elevations of the cuticula over the ventral and lateral surfaces of the outside border. Pharynx oval; length equal to, or greater than diameter of oral sucker. Walls of esophagus somewhat irregular, narrow anteriorly but usually much inflated near bifurcation. Ceca compressed laterally throughout most of length and extend to posterior end where they are attached to, or at least in contact with, the terminal chamber of the excretory bladder. Acetabulum spherical, near middle of body; diameter about twice that of oral sucker. Crown wedge-shaped, bearing 20 relatively large spines; arranged 9 on either side in a single row that is interrupted dorsally, and 1 on each angle. Angle spines usually much smaller than others but are sometimes almost equal to them; margin spines nearly uniform in size and shape.

Testes contiguous, round to elongate transversely; anterior testis typically wider and usually larger than posterior one; margins simple. Ovary on right side; diameter varies from slightly less than, to 1.5 times the length of ova. Oviduct arises dorsally and enlarges to form a distinct fertilization chamber just proximal to junction of Laurer's canal; Laurer's canal long, containing sperms in inner portion; outer portion very narrow and apparently without opening to outside; attachment to body wall directly dorsal to left intestinal cecum. Yolk reservoir voluminous, receiving right and left vitelline ducts separately. Yolk ducts pass ventral to ceca in reaching vitellaria. Vitelline follicles extend from level of acetabulum to posterior end, the lateral fields joined back of testes by 5 or 6 isthmuses ventral to the excretory bladder and 3 or 4 dorsal to it. Uterus short, forming 3 to 6 horizontal and vertical loops; sperms stored in posterior portion of uterus. True seminal receptacle absent. Metraterm on left side and somewhat ventral to cirrus sac. Genital atrium shallow, simple. Cirrus sac ovoid, usually widest at its anterior end; mostly dorsal to acetabulum, usually extending far beyond middle of acetabulum and sometimes reaching its posterior border. Seminal vesicle voluminous, divided into posterior and anterior chambers. Pars prostatica and prostate gland small but distinctly present. Cirrus extremely short and simple. Genital pore crowded between acetabulum and gut bifurcation. Excretory bladder divided into a longitudinal series of 5 chambers; terminal chamber lined with irregular cuboidal epithelium which is continuous with the intestinal ceca although apparently there is no communicating lumen between the two structures. Lateral excretory siphons large in preacetabular region. Measurements on average specimen: length, 1.51 mm; width at level of crown, pharynx, acetabulum, and anterior testis respectively, 0.248, 0.170, 0.256, and 0.240 mm; oral sucker, 70 by 65  $\mu$ ; pharynx, 80 by 60  $\mu$ ; diameter of acetabulum, 153  $\mu$ ; cirrus sac, 160 by 84  $\mu$ ; diameter of ovary, 70  $\mu$ ; eggs, 72 by 54 to 76 by 50  $\mu$ ; anterior testis, 120 by 154  $\mu$ ; posterior testis, 118 by 146  $\mu$ ; spines on margin of crown, 66 by 14 to 72 by 15  $\mu$ ; angle spines of crown, 40 by 10  $\mu$ ; length of cuticular spines at level of genital pore, 10  $\mu$ .

*Host:* *Podilymbus podiceps*.

*Habitat:* Duodenum.

*Locality:* Indian River, Michigan, U. S. A.

*Paratype specimens:* U. S. Nat. Mus. Helm. Coll. Nos. 36723-36725.

#### DISCUSSION

The cercaria of *Echinochasmus donaldsoni* is closely related to *Cercaria ornatostoma* Cable, 1935. The behavior and general shape of the body, suckers, and tail are similar; cuticular and collar spines are lacking in both; cystogenous glands consist of angular masses of small slender rods; and the excretory bladder, ducts, and granules are identical in pattern. Oral spines occur on the ventral and dorsal lips of the oral sucker, and similar spines form a single row on the margin of the acetabulum. The rediae of the two species are likewise similar in the character of the collar, appendages, birthpore, and sucker; the gut is long in both. *C. ornatostoma* is much the larger of the two species, moderately extended specimens measuring 0.2 mm in length while specimens of the cercaria of *E. donaldsoni* are only 0.12 mm when fully extended. The oral sucker of *C. ornatostoma* bears a ring of about 12 large perioral cells that are not found in the other species, and the oral spines of the ventral lip are 8 in *C. ornatostoma* instead of 10. Siphon granules are preacetabular in *C. ornatostoma* while they extend into the postacetabular region in the other. *C. ornatostoma* was found in *Goniobasis semicarinata* in Kentucky.

There are 27 more or less well known species which have been placed in the genus *Echinochasmus* Dietz, 1909. In 1931, Price reviewed the genus and listed the known species. Since then the following species have been added: *E. novalichensis* by Tubangui (1932); *E. rugosus* and *E. redioduplicatus* by Yamaguti (1933); *E. ruficollis* by Ishii (1935); *E. ruficapensis* and *E. bagulai* by Verma (1935); *E. narayani* by Mudaliar (1938); *E. gorsakii*, *E. milvi*, and *E. tobi* by Yamaguti (1939); and *E. reniovarus* and *E. megavitellus* by Lal (1939). Because the vitellaria in *E. reniovarus* extend anteriorly into the preacetabular region, it must be removed from the genus *Echinochasmus* and placed in the genus *Episthmium* Lühe, 1909. It therefore becomes *Episthmium reniovarus* (Lal, 1939).

The numerous species of the genus *Echinochasmus* form 4 sub-groups, based on the number of collar spines. Four species, all Japanese, have 28 or more collar spines; 15 species have 24; 6 species have 22; and *E. dietzevi* Issaïtschikoff, 1927, like the form described here, has but 20 collar spines.

Comparison of *E. dietzevi* with the 20-spined North American form reveals a number of striking differences if the description of the former may be regarded as accurate for typical normal specimens. *E. dietzevi* has 3 alternating angle spines while *E. donaldsoni* has 1 only. Back of the

testes, the vitellaria form rather solid fields that almost meet at the mid-ventral line and are confluent dorsally in *E. dietzevi*. In *E. donaldsoni* the vitellaria of the two sides are joined by isthmuses along both the dorsal and ventral sides. The intestinal ceca extend almost to the body wall at the posterior end in *E. dietzevi*, leaving no space for an inflated portion of the terminal part of the excretory bladder such as is found in *E. donaldsoni*. Except for the differences in spine pattern, the other differences might easily be explained as due to relatively poor specimens, or lack of detail in description. There is perhaps still some question as to whether distinct species of echinostomes may have identical spine pattern, but there cannot be the least doubt that individual collections of worms with different collar spine patterns are distinct species.

Although the life cycle of *E. perfoliatus* (Ratz) is somewhat imperfectly known (Muto, 1921), apparently it is the only species of the genus whose development, modes of transmission, and hosts are known to closely resemble those of *E. donaldsoni*. The cercariae of both are minute gymnocephalous forms which differ principally in the distribution of the siphon granules and pigmentation of the cystogenous gland cells. In both species the cercariae are carried into the pharynx of freshwater fish where they encyst on the gill filaments, and when infective metacercariae are eaten by the final host, maturity in the adult stage is reached in a relatively short period, 7 to 10 days. The metacercariae of *E. japonica* Tanabe (Ujiie, 1936) and *E. liliputanus* (Looss) (Ciurea, 1931) also are found on the gills of freshwater fish.

#### SUMMARY

1. A new gymnocephalous cercaria is described from *Annicola limosa* and *A. lustrica* from the region of Douglas Lake, Michigan, and its life cycle demonstrated experimentally.
2. The cercariae encyst on the gills of numerous species of small freshwater fish and develop collar spines typical of echinostomes.
3. Metacercariae are infective after 3 weeks and become adults in the duodenum of pigeons, maturing on the 7th day.
4. The pied-billed grebe (*Podilymbus podiceps*) is a field host for the adult.
5. The adult is described as a new species, *Echinochasmus donaldsoni*.

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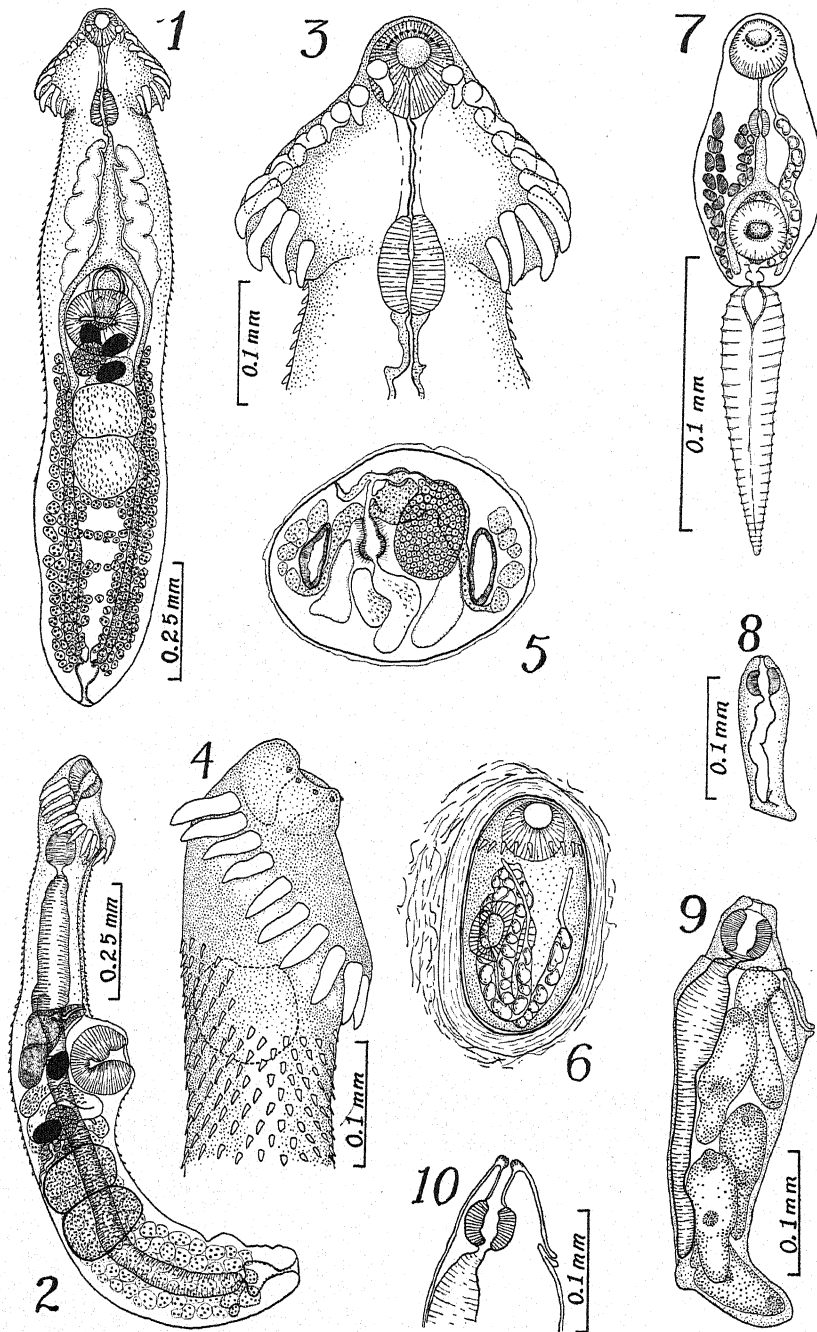


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## EXPLANATION OF PLATE

Drawings made with the aid of a camera lucida.

- FIG. 1. Young adult. Ventral view.
- FIG. 2. Young adult. Lateral view.
- FIG. 3. Head crown of adult. Ventral view.
- FIG. 4. Head crown of adult. Lateral view.
- FIG. 5. Composite transverse section of mature worm at level of ovary, fertilization chamber, Laurer's canal, ootype, etc. Anterior view.
- FIG. 6. Metacercaria, 4 weeks after encystment.
- FIG. 7. Cercaria. Ventral view, moderately contracted. (Outlines drawn from fixed specimen.)
- FIG. 8. Immature redia. Ventro-lateral view.
- FIG. 9. Mature redia. Lateral view, moderately contracted.
- FIG. 10. Anterior end of mature redia showing pharynx withdrawn to level of birthpore and the collar constricting the opening at the extreme anterior tip.



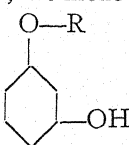


# ANTHELMINTIC STUDIES ON THE MONO ETHERS OF THE DIHYDROXY BENZENES

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The anthelmintic activity of the resorcinols and their derivatives has been studied extensively in recent years, particularly by Lamson and his associates. As is well known the most desirable member of this class of compounds, viewed from the standpoint of both anthelmintic activity and toxicity, is hexylresorcinol. There is another group of closely related compounds, however, the mono ethers of the dihydroxy benzenes, having

the general formula  whose anthelmintic activity has not been investigated as far as the authors are aware.

Some of the members of this class of compounds occur naturally (guaiacol, beechwood creosote, oil of clove), while others (resorcinol, hydroquinone, catechol) must be prepared synthetically. Although it is recognized that many more compounds of this series exist, only the compounds having a normal arrangement of carbon atoms from  $C_4$  to  $C_6$  were prepared since this range has been demonstrated by other investigators to be the most effective as anthelmintics. The organic synthesis of these members was carried out by one of us (C. F. L.) and will not be considered here in detail. It is sufficient to say that the compounds had the proper structure and the degree of purity required by the accepted standards.

## EXPERIMENTAL METHODS

The anthelmintic activity of the compounds studied was determined by the *in vitro* method of Lamson and Brown, the mechanics of which have been previously described (Landsberg and Accousti (1)). The test object, *Ascaris lumbricoides* of swine, was obtained at a local slaughter house. The worms were exposed to the drug and then tested for motility, by dropping into water at  $60^\circ C$ , twenty-four hours after exposure. If no motion in either direction was observed they were considered dead.

To determine the toxicity of the compounds studied the usual pharmacological procedures were carried out. As a preliminary measure graduated doses, based on grams per kilo body weight, were given to adult rats by stomach tube. By this method the dose which would kill in twenty-

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four hours was determined. This dose then was administered to each of 10 rats and the mortality in twenty-four hours determined. Since the objective was to determine the dose which would produce a fifty per cent mortality (L. D. 50) in twenty-four hours, this dosage was raised or lowered until that quantity of drug was found. In some instances this involved several groups of 10 rats each. When the fifty per cent killing dose was established a final 10 animals were used for confirmation.

#### EXPERIMENTAL RESULTS

As a preliminary experiment runs were made on the various mono ethers using two ascarids for each run. The concentration used in each instance was one to a thousand. The results have been summarized in Table 1.

TABLE 1.—*Preliminary runs on the mono ethers of the dihydroxy-benzenes*

Compound*	Time								
	2'	5'	10'	20'	30'	45'	1 hr	2 hr	3 hr
	Percentage of worms killed								
Resorcinol mono-n butyl ether ..	0	50	100	100	100	100	100	100	100
Resorcinol mono-n amyl ether ..	0	100	100	100	100	100	100	100	100
Resorcinol mono-n hexyl ether ..	0	0	100	100	100	100	100	100	100
Hydroquinone mono-n butyl ether	0	0	66	100	100	100	100	100	100
Hydroquinone mono-n amyl ether	0	0	33	100	100	100	100	100	100
Hydroquinone mono-n hexyl ether	0	0	50	100	100	100	100	100	100
Catechol mono-n butyl ether ...	0	0	0	0	0	0	0	100	100
Catechol mono-n amyl ether ...	0	0	0	0	0	0	50	100	100
Catechol mono-n hexyl ether ...	0	0	0	0	0	50	100	100	100
Gualiacol .....	0	0	0	0	0	50	100	100	100
Beechwood Creosote .....	0	0	0	0	50	50	100	100	100
Iso Eugenol .....	0	0	0	0	0	0	100	100	100
Eugenol .....	0	0	0	0	0	0	100	100	100
Oil of Clove .....	0	0	0	0	0	50	50	100	100

\* Concentration 1:1,000.

From this table it may be observed that resorcinol mono-n butyl ether, resorcinol mono-n amyl ether and resorcinol mono-n hexyl ether had the best anthelmintic activity. The first killed fifty per cent in 5 minutes, the second killed one hundred per cent in 5 minutes and the third killed one hundred per cent in ten minutes.

Since only two worms were used in the preliminary runs, other runs were made on the three compounds showing the most promise, using larger numbers of ascarids. In each instance 12 worms were removed at each time interval, and as all the worms were killed after twenty minutes of exposure, the experiment was terminated after one-half hour.

From Table 2 it may be observed that resorcinol mono-n hexyl ether gave the best results, killing fifty-five per cent of the ascarids in 2 minutes and one hundred per cent in 5 minutes. At this time it should be pointed out how the additional number of worms shifted the killing power of the



TABLE 2.—Comparison of some of the mono ethers of dihydroxy-benzene with hexylresorcinol

Compound*	Time in minutes				
	2	5	10	20	30
	Percentage of worms killed				
Resorcinol mono-n butyl ether .....	0	10	73	100	100
Resorcinol mono-n amyl ether .....	40	42	73	100	100
Resorcinol mono-n hexyl ether .....	55	100	100	100	100
Hexylresorcinol .....	75	100	100	100	100

\* Concentration 1:1,000.

compound. When only two worms were run, resorcinol mono-n amyl ether appeared to have the most activity, killing one hundred per cent in 5 minutes (Table 1), and resorcinol mono-n hexyl ether appeared to have less activity than any of these three compounds, not killing one hundred per cent until ten minutes (Table 1). When 12 worms were removed at each time interval, however, using the same concentration, the killing ability of the compounds was shifted markedly. In this instance resorcinol mono-n hexyl ether showed the most activity, killing one hundred per cent in five minutes (Table 2) while resorcinol mono-n amyl ether did not kill one hundred per cent until 20 minutes exposure (Table 2).

It is recognized that hexylresorcinol has probably the best anthelmintic activity of this general class of compounds. With this in mind the three compounds prepared by us were compared with hexylresorcinol by the same in vitro method. The results of this comparison have been summarized in Table 2. It may be observed from this table that the anthelmintic activity of resorcinol mono-n hexyl ether is closer to that of hexylresorcinol than any of the other compounds of this series. On the basis of 12 worms removed at each stated time interval resorcinol mono-n hexyl ether killed fifty-five per cent after two minutes exposure as compared with seventy-five per cent killed by hexylresorcinol for the same period under the same experimental conditions. At the five minute period, however, the two compounds had equal killing power, both killing one hundred per cent.

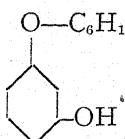
Since the in vitro anthelmintic activity of resorcinol mono-n hexyl ether and hexylresorcinol is very close, varying only twenty per cent at two minutes exposure (Table 2), it appeared desirable to determine the relative toxicity of these two compounds. One of the common pharmacological procedures, used for the determination of an acute dose, is the establishment of the L. D. 50 for twenty-four hours, i.e., the dosage which will kill fifty per cent of the experimental animals in twenty-four hours.

From a preliminary study on rats it was found that 1.75 gm resorcinol mono-n hexyl ether per kilo of rat, killed in twenty-four hours. This dosage, after further testing in other rats, was found to be the L. D. 50 for twenty-four hours. When the same dosage of hexylresorcinol (1.75 gm per kilo) was given to rats the killing power was essentially the same. On the basis of toxicity in rats then, resorcinol mono-n hexyl ether does not appear to be more toxic than hexylresorcinol.

#### DISCUSSION

On the basis of the experimental procedures outlined previously, resorcinol mono-n hexyl ether has the best anthelmintic activity of the group of fourteen mono ethers of the dihydroxy benzenes studied. In passing it may be observed also that of these compounds the synthetic members have greater anthelmintic activity than those occurring in nature (Table 1). By the same in vitro testing method resorcinol mono-n hexyl ether appears to have about the same anthelmintic activity as hexylresorcinol. The toxicity of this compound in the rat on a grams per kilo basis compares favorably with hexylresorcinol.

Resorcinol mono-n hexyl ether is a reddish-brown, oily liquid having

the formula . Its solubility is close to that of hexylresor-

cinol. Lamson et al. (2) in describing the physical properties of a phenol required for good ascaricidal activity, maintain that it should be a liquid and have a solubility ranging from 1:1,000 to 1:35,000. It may be observed that resorcinol mono-n hexyl ether falls into such a category. It is realized fully that the activity observed in the in vitro ascaris testing procedure does not give an absolute index of anthelmintic activity in either man or other animals, but in view of the observations it appears to the writers that this compound, resorcinol mono-n hexyl ether, merits additional investigation. Further studies should include the in vivo action on the dog ascaris and the toxicity of the compound for canines with the resultant pathology. Upon the basis of these results the efficacy of the compound for other animal parasites, as well as for human administration, may be considered.

#### SUMMARY

1. Of the fourteen compounds of the mono ethers of dihydroxy benzene, resorcinol mono-n hexyl ether appears to have the greatest anthelmintic activity when tested by the in vitro ascaris technique.
2. The toxicity of this compound, when tested on rats, is no greater than that of hexylresorcinol.

3. In the opinion of the authors this compound merits further investigation, particularly in vivo anthelmintic and toxicity studies in dogs.

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## RESEARCH NOTES

### OCCURRENCE OF *TRICHOSTRONGYLUS COLUBRIFORMIS* IN THE GUINEA PIG

A male specimen of *Trichostrongylus colubriformis* 4.5 mm long was discovered in the posterior part of the small intestine when a young guinea pig, experimentally infected with *Trichinella spiralis*, was examined for adult trichinae. Autopsy of another young guinea pig, purchased at the same time and used in the same experiment, did not reveal any trichostrongylids, nor could helminth eggs be demonstrated in a fecal sample from its rectum.

Altogether, of a large number of trichinized guinea pigs whose intestines have been examined for adult trichinae, this was the only case in which accidental infection with another worm was met with. The guinea pig in question had been purchased from a man who bought up laboratory animals from small landowners in the vicinity of Copenhagen. The animal must be supposed to have picked up the infective larva when it was fed with fresh grass from an area where infected sheep were running. Then, the very young age (few months) of the guinea pig may have been one of the causes why such an unusual parasite could reach maturity in its intestine.

*T. colubriformis*, a very common parasite of the sheep, has also been reported from a long series of other hosts, as goat, cattle and other ruminants, horse, squirrel, hare, rabbit, zizel, several kinds of apes and monkeys, and man.—HANS ROTH, *Institute for General Pathology, University of Copenhagen* (Director: Professor Oluf Thomsen).

### NOTE ON LIFE HISTORIES OF THE GENUS *PARAMONOSTOMUM* LÜHE, 1909 (TREMATODA: NOTOCOTYLIDAE) WITH SPECIAL REFERENCE TO THE EXCRETORY VESICLE

During the past five years repeated efforts have been made to solve the life-histories of the notocotylid cercariae parasitizing *Peringia ulvae* (Pennant, 1777). The experiments have been tried again this winter with partial success.

Six species of these larvae were isolated and the cysts fed to laboratory reared ducklings. Three pertained to the Monostomi sub-group and three to the Yenchingensis sub-group. Although the material of the former group was far more plentiful all efforts at rearing the adult worms again failed. Two species of the Yenchingensis sub-group, however, developed in the intestinal ceca of the ducks, into flukes of the genus *Paramonostomum*.

It is well known that in mature notocotylid trematodes the excretory vesicle becomes greatly complicated. The essential change lies in the development of numerous large ramifying branches arising from the lateral edges of the vesicle. This system is not visible in preserved material and has only been figured by Looss (1896, Mem. Inst. Egypt. 3: 1-252) for *Catantropis verrucosa* (Fröl. 1789) and by Noble (1933, Tr. Am. Micr. Soc. 52: 353-359) for *C. pacifera* Noble, 1933. In these drawings there is no structure shown which could represent the unpaired diverticula present in Yenchingensis cercariae. In this region (the extreme anterior portion of the vesicle) there are a pair of branches figured, one lying on either side of the esophagus.

The excretory system of the adult *Paramonostomum* is very similar to that of *Catantropis*; large branches developing along the lateral edges of the vesicle. These are difficult to trace to their extremities, but in some very favorable specimens it appears that the branches arising from the inner margin anastomose in the center line of the body, thus forming a network. The median unpaired diverticula of the cercaria persists as a large conspicuous unpaired branch immediately overlying the esophagus (Figs. 1 & 2).

It will be recalled that in an earlier publication (Rothschild, 1938, Novit. Zool. Tring. 41: 75-83) the author divided the notocotylid cercariae into three groups



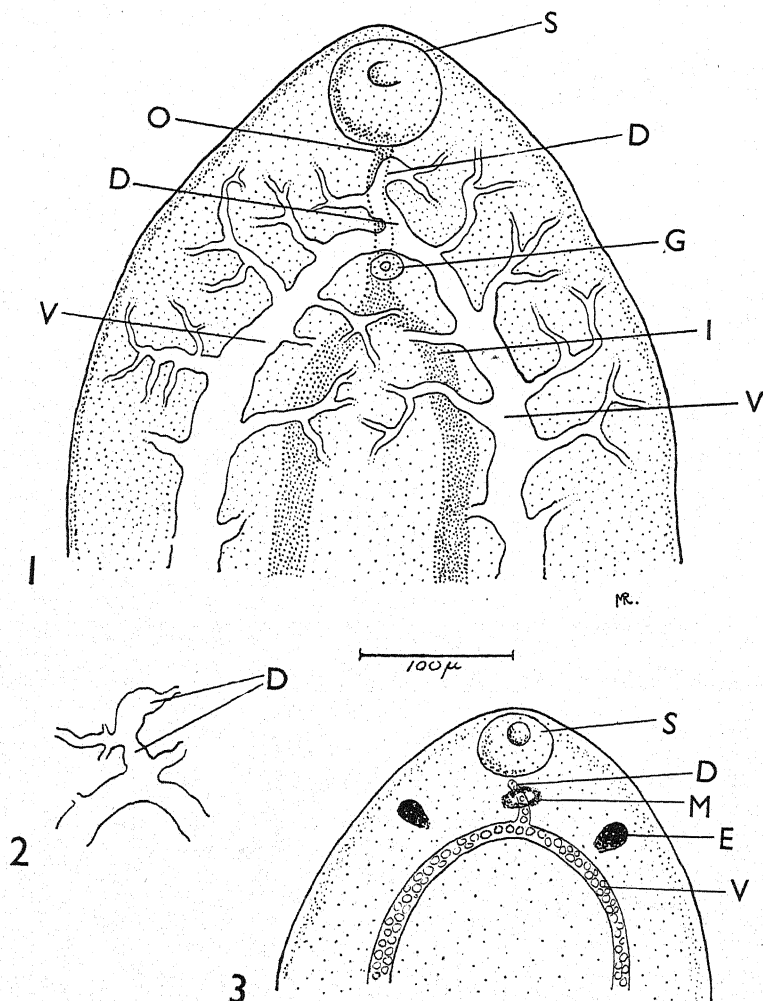


FIG. 1. Anterior end of *Paramonostomum* sp. showing the excretory vesicle. The diagram was drawn with the aid of a camera lucida during life and the finer branches are omitted. Anastomosing of the median branch could not be made out in this specimen.

FIG. 2. The unpaired diverticula in another specimen.

FIG. 3. Anterior end of the cercaria of *Paramonostomum* sp. (Yenchingensis sub-group) showing the unpaired diverticula of the excretory vesicle. (Camera lucida drawing.)

#### ABBREVIATIONS

- D—unpaired diverticula of excretory vesicle
- E—lateral eye spot
- G—genital pore
- I—intestine
- M—median eye spot
- O—esophagus
- S—oral sucker
- V—excretory vesicle

according to the structure of the anterior portion of the excretory vesicle. (It is probable that a fourth group may be now added in which the two halves of the excretory vesicle do not fuse in the anterior region, but remain separate, as in the earlier stages of cercarial development. This is suggested by Herber's interesting experiments (Herber, 1939, J. Parasitol. 25: Suppl: 18-19.) and some unpublished drawings shown to the author by Prof. R. P. Dollfus. This character will probably be found to characterize the adult worm as well as the larva.) It is therefore of interest to find that the unpaired diverticula of the cercaria (Fig. 3) persists in the adult *Paramonostomum*. However, the genus *Notocotylus* as it now stands may well contain two or more types of excretory vesicle. Thus at least one cercaria of the Yenchingensis group and one of the Monostomi group (Szidat, L. and U., 1933, Centr. Bakt. I Abt. Orig. 129: 411-122; Yamaguti, 1938, Z. Parasitenk. 10: 288-292) have been reported to develop into the same species of adult, *Notocotylus attenuatus* (Rud. 1809)!

Further investigation into the excretory system of adults of the Monostomi subgroup is therefore very desirable and renewed efforts are being made to rear those species found in *Peringia ulvae*.—MIRIAM ROTHSCHILD, *Peterborough, England*.

#### THERMAL DEATHPOINT OF THE ERYTHROCYTIC STAGES OF *PLASMODIUM CIRCUMFLEXUM*

Very little work has been done on the thermal deathpoint of the malaria parasite. In their paper on cultivation Bass and Johns (1912, J. Exper. Med. 16: 567) stated that glass pipettes recently flamed should be allowed to cool below 50° C since otherwise the blood and parasites might be injured. The present study was undertaken since we needed to know the thermal deathpoint of *P. circumflexum* in the erythrocytic cycle in connection with immunity experiments.

Two strains of *P. circumflexum* were used. In earlier work (Manwell and Goldstein, 1940, J. Exper. Med. 71: 409) these were designated as Strains E and A, and a history of the strains was presented. Both strains are virulent and show exo-erythrocytic stages with regularity.

The technique used to determine the deathpoint was of two sorts. At first the parasites were placed in a constant temperature water bath. But it was found that results obtained this way did not differ markedly from those made by placing the blood in a constant temperature incubator equipped with a De Khotinsky thermal control. Since this latter method was simpler, it was used in most of the experiments. The vial containing the blood and parasites was then placed in a vessel filled with water at the same temperature as the incubator. This step was to prevent any changes of temperature which might be produced by opening and closing the incubator door.

The blood was removed with sterile precautions from a heavily infected canary and mixed with either citrated physiological saline, or saline to which heparin had been added. This was then placed in a glass vial and sealed air-tight with a sterile piece of rubber dam, after taking pains to remove all of the sulphur bloom from the rubber. The vial was now put into the hot water bath or into the incubator. In all experiments, two minutes were given for the glass vial and fluid to reach the experimental temperature. Every five minutes the vials were shaken.

A total of 47 female canaries was used. Of this number, 36 were experimental while 11 served as controls. 33 were Strain E cases; 3 Strain A. All injections of previously heated material were intravenous.

The results are summarized in Table 1.

It is clear from these data that the thermal deathpoint of erythrocytic stages of *P. circumflexum* is 50° C for 15 minutes of exposure; or at 55° C for 5 minutes of heating. This assumes, of course, that sufficient time has been allowed for the warming of the vial and fluid to the experimental temperature. It would be difficult to establish the exact point of death and a more complicated technique would have to be employed to this end. But for general purposes, the above range is sufficiently accurate. At 40° C there was no effect of temperature on the parasites even when ex-

posed 30 minutes. At 45° C there was a delayed prepatent period over that of the controls of from 3 to 6 days in samples exposed longer than 15 minutes. This is indicative that some of the parasites were killed by the heating while others resisted it, or that the parasites were weakened but recovered. At 50° C any exposure over 15 minutes resulted in no infection, while 5 and 10 minute exposures caused a delay similar to that observed at 45° C for 15 minutes. At 55° C five minutes of exposure was sufficient to kill all of the parasites.

TABLE 1.—Effect of temperature on prepatent period and parasites

Exposure time (minutes)	40° C. (1 case each)	45° C. (2 cases each)	50° C. (3 cases each)	55° C.
5	No effect	No effect	Delayed	No infection (12 cases)*
10	No effect	No effect	Delayed	No infection (3 cases)
15	No effect	Delayed	No infection	No infection (5 cases)
30	No effect	Delayed		

\* 9 cases of Strain E and 3 cases of Strain A.

An interesting fact is that blood heated to 55° C for even 15 minutes shows little morphological change when smeared and stained with Giemsa. Naturally the color had darkened much during exposure to the heat. The parasites in such blood show very little change in shape other than a tendency to round up. The main apparent change in the parasites occurs in the staining since they appear darker in color, both the chromatin and cytoplasm. But merozoites look very much like normal ones except, again, that they stain more darkly.

*Conclusions:* The thermal deathpoint of *P. circumflexum* in red blood cells appears to be 50° C when exposure is for 15 minutes, or 55° C for 5 minutes approximately. There is no hemolysis of the blood at these temperatures. The parasites undergo little morphological change other than a tendency to round up and take a deeper stain with Giemsa.—FREDERICK COULSTON, *Department of Zoology, Syracuse University.*

#### BALANTIDIUM COLI AND PINWORM IN A CHIMPANZEE

In 1935 a young chimpanzee in the Chicago Zoological Park at Brookfield, Illinois, was reported by its attendant to be ailing. A fecal sample was sent to the writer. The sample contained considerable mucus but was formed. Microscopic examination revealed a moderate *Balantidium coli* infection.

Five days later another fresh sample was obtained. It was fluid with much mucus. One active female pinworm was found, as well as many eggs. The number of balantidia had greatly increased over that in the previous sample. Both cysts and trophozoites were plentiful.

The above is a record of a double infection, helminthic and protozoan. The correlation between the diarrhetic condition of the fecal sample and the increase in the number of balantidia is marked. Hegner (1934, *Am. J. Hyg.* 19: 480-501) states that in fecal examination of fifteen chimpanzees the impression was gained that the animals most heavily infected with balantidia had the greatest tendency to become diarrhetic. The presence of the pinworm may have been a factor contributing to the diarrhea in the case of the Brookfield chimpanzee.—ALBERT E. MOORMAN, *Kansas Wesleyan University, Salina, Kansas.*

## REVIEW

CARLOS FINLAY AND YELLOW FEVER. By Carlos E. Finlay. xii + 249 pp., 25 illustrations. Oxford University Press, New York. 1940.

No short review can do justice to this attractive volume, which brings together biographical notes on Carlos Finlay, the story of the development of his theory of mosquito transmission of yellow fever, the record of the results obtained in the control of yellow fever following the confirmation of his theory in 1900, and a discussion of recent laboratory and epidemiological findings in the light of his experimental work.

The purpose of the volume is best stated in the author's own words:

"The misconception of my father's role in the discovery of the transmission of yellow fever by the mosquito, its undervaluation and the nonrecognition of the experimental phase of the work, which has prevailed in almost all the discussions of yellow fever in the English language, constitute the principal inducements which have led me to write this book."

This labor of filial love has been carefully executed and the result is a most excellent brief, brought up to date on the basis of laboratory and field work during 1927 and 1940, for the plaintiff in the case of *Finlay vs Reed et al, and Gorgas*: by a coincidence it has the same number of pages as has the Senate Document No. 822 (1911), which may be considered as the brief for the defense. The two volumes should be studied together.

It is indeed regrettable that unpleasant discussions as to who should get the credit for the mosquito transmission of yellow fever should have occurred; surely there was glory and to spare for all who had any part in contributing to the work which led to the control of the most fearful of tropical scourges.

Such discussions were, however, unavoidable since Finlay believed so utterly in the doctrine of mosquito transmission that "... it is a question if his faith in it was increased by the results of Reed's experiments" (Carter), but was unable to convince others; "... in the trying period from 1881 to 1900 only two figures stand forth in the background: one was my mother, his wife and faithful helpmate . . . : the other was Dr. Claudio Delgado, at that time my father's only disciple. . . ." In spite of exceptional opportunities to present his theories before scientific societies in Cuba, the United States and Europe, and personally to such students of the disease as Sternberg and Gorgas, Finlay never succeeded in convincing others of the validity of his experimental work. Reed and Carroll, who became part of the Army Commission in Cuba in 1900, had been working on the etiology of yellow fever since 1897 and turned to the theory of mosquito transmission only after all other leads had been played out and after Carter's field observations indicated the necessity for an "extrinsic incubation period" between the first case and secondary cases in any community.

It is perhaps natural that advantage should have been taken of these discussions by strong nationalists and others with political axes to grind:

"... those flocking after him because of his theory did so from patriotic and other motives which before had not appealed to them" (page 25).

One cannot but consider as suspect such actions as that cited on page 44:

"Finally, at the closing session of the German Ibero-American Academy held at Vienna in August, 1939, a resolution was unanimously carried recognizing *Carlos J. Finlay as the sole discoverer of the mosquito transmission of yellow fever.*"

It is hardly to be expected that the historians of the future will accept this sweeping conclusion with its implications of criminal negligence of health officers who permitted the uncontrolled ravages of yellow fever from 1881 to 1901.

Surely, it will not be difficult to reconcile rival claims in the face of (1) Reed's declaration, page 219:

"We here desire to express our sincere thanks to Dr. Finlay who accorded us a most courteous interview and has gladly placed at our disposal his several publications relating to yellow fever, during the past nineteen years; and also for ova of the species of the mosquito with which he has made his several inoculations . . . with the mosquitoes thus obtained we have been able to conduct our experiments."

and (2) Finlay's statements in 1903:

*"I have never claimed that I had demonstrated the factors, but I have proved by unmistakable evidence, in print, that I had foretold the results which the Army Board afterwards confirmed, giving them as original discoveries of their own."* (The transmission of yellow fever. J. Am. Med. Assn., 1903, p. 1660).

It is to be expected that the final decision will be in accord with the tribute paid by Doctor Sawyer on his reception of the "Carlos J. Finlay" decoration May 16, 1940, which forms the final paragraph of the Appendix of the book under review:

"The decoration which I am receiving at your hands might appropriately be considered a recognition of the yellow fever research of my colleagues as well as my own, for we always work as a group, and I am especially pleased to learn that one of them, Dr. Fred L. Soper, is being similarly honored on this occasion. The decoration is especially dear to me, as a student of yellow fever, because it bears the name and likeness of Carlos Finlay, who made the first great contribution to our knowledge of the disease. Far ahead of his time, and at the very beginnings of bacteriology, he published a hypothesis that yellow fever was transmitted by a special mosquito and strove for two decades to prove his thesis, experimentally to the satisfaction of his contemporaries. The fact was finally firmly established for him, and with his friendly collaboration, by the Army Commission. During the long period in which his thesis was refused acceptance and was even scorned, he held firmly to his convictions and at last he was able to see the full acceptance of his theory and its application to the immense benefit of man. In accepting this decoration I pay homage to Carlos Finlay."

Attention should be called to the fact that the Bibliography as given fails to cite the following publications:

- 1888 The microbe of yellow fever. Medical Record, New York, 33: 622.
- 1889 Sur le Micrococcus versatilis (Sternberg) C. Delgado and C. Finlay J. Anat. et Physiol. 25: 223.
- 1896 Apéndice á la comunicación sobre el tetracoca versatilis y la fiebre amarilla. 8 pp. 8°, Habana.
- 1899 Work cited by Reed. Medical Record, New York, 55(21). May 27, 1899.
- 1900 Etiología de la infección hemogástrica en la fiebre amarilla. Rev. Med. y Cir., Habana, 5: 117-122. Also 1899, An. Acad. 36: 183-190.

—FRED L. SOPER, Rio de Janeiro, Brazil.



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## STUDIES ON THE GAPEWORM *SYNGAMUS TRACHEA* (MONTAGU, 1811) IN ROBINS AND CHICKENS<sup>1,2</sup>

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Manter and Pinto (1928) described *Syngamus tenuispiculum* from the robin in Nebraska but indicated that possible variations in the chicken gapeworm (*Syngamus trachea*) might show that the robin gapeworm is also *S. trachea*. The studies reported below were intended to determine the relationship between the robin and chicken gapeworm.

*Syngamus trachea* has been reported from the robin by Walker (1886). Walker also pointed out that the larvae of *S. trachea* were present in earthworms, and showed that earthworms were not a necessary host but could produce the disease in chicks that fed upon them. Walker experimentally infected robins with gapeworms from the chicken by feeding three young robins in the nest several infected earthworms. On examination, two of the robins were found to harbor three or four gapeworms each. In another experiment a young robin just from the nest was fed a large number of larvae hatched in water. It was kept in a cage hanging under a tree and fed by the old bird. On examination, three fair sized syngami were found in the trachea. The experimental infections of robins—as done by Walker—were incidental to attempts to prove the importance of earthworms in the transmitting of gapeworms to chickens, and the experiments were not well enough controlled to be conclusive.

*S. trachea* is known from various wild birds in England and the starling apparently is of considerable importance in the spread of the disease among domestic poultry in that country. Lewis (1926) showed that starlings play an important rôle in the distribution of gapeworms in chickens, while Taylor (1928) contended that the eggs obtained from the gapeworm of starlings have only a low infectivity to young chickens, but

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<sup>1</sup> Studies from the Zoological Laboratories, University of Nebraska, No. 207.

<sup>2</sup> The present studies were made under the direction of Dr. H. W. Manter. The author is also indebted to Dr. Benjamin Schwartz of the U. S. Bureau of Animal Industry for sending specimens of *Eisenia foetida* infected with *Syngamus trachea* from the chicken.

"once an infection has been obtained the second generation enters the chick readily." Clapham (1935) showed quite conclusively—by using the indirect method of infection—that the starling strain of gapeworms is very infective to chickens. Rice (1929) transmitted a gapeworm indistinguishable from *S. trachea* from the rook (*Corvus frugilegrus*) to the chicken.

Cram (1927) gave a list of 28 species of wild birds in which "gapeworms identical in structure with *Syngamus trachea* have been obtained." The record of *S. trachea* from the robin in America by Walker (1886) and Megnin's (1883) report of its occurrence in the swift were not included in her list. In addition to these hosts, *S. trachea* has also been reported from the sparrow, linnet, martin, kestrel, pigeon, and sandpiper (Campbell, 1935), and from Gambel's sparrow, water thrush, and junco (Cram, 1931). Cram noted the common occurrence of the parasite in wild birds in Alaska where it has not been reported from the chicken.

In this country, *S. trachea* has been reported as occurring naturally in turkey, chicken, robin, bobwhite quail, ruffed grouse, and pheasant. The possible rôle played by wild birds in the dissemination of gapeworms in the United States is unknown.

It is known that the life cycle of *S. trachea* can be completed with or without an earthworm but usually a higher percentage of infections as well as a larger number of worms are secured when infected earthworms are fed to experimental chicks. To Waite (1920) belongs the credit for establishing the fact that earthworms are an important factor in the transmission of gapeworms—as indicated by Walker. Clapham (1934) found more certain and more severe infections through the use of *Eisenia foetida* than when other species of earthworms were used.

Of 76 robins collected at Lincoln, Nebraska, 25 or about 33 per cent were infected with gapeworms. Usually only one pair of worms was present but one robin contained three pairs. The worms occurred all along the trachea but usually in the lower third.

#### MORPHOLOGY

The morphology of these worms from the robin may be briefly described as follows (measurements in millimeters):

*Male*: Length, 1.146 to 4.239 (average of 31 specimens, 2.631). Buccal capsule, 0.189 to 0.288 in external diameter (average 0.209), with cuticular rim. Esophagus, 0.503 to 0.706 (average 0.601). Dorsal ray cleft, each branch usually bidigitate, sometimes tridigitate (in one specimen, one branch was bidigitate, the other unforked); externo-dorsal ray rather short and thick; lateral rays about equal and close together; ventral rays similar. Spicules slender, usually subequal, rarely equal, 0.608 to 0.805 in length.

*Female*: Length, 6 to 14 (average of 31 specimens, 9.5); width, 0.320 to 0.735. Vulva 2.19 to 3.50 from the anterior end. Buccal capsule, 0.193 to 0.278 in external diameter (average, 0.235), with cuticular rim. Esophagus, 0.518 to 0.785 in length

(average 0.611). Number of teeth varying from seven to nine in both sexes, usually eight; tendency toward nine teeth in the female and seven in the male. Eggs average 0.090 by 0.047.

This gapeworm appears to differ from *S. merulae* in the presence of a buccal rim and different dorsal rays. There seem to be no morphological grounds for separating gapeworms of the robin and chicken, and *S. tenuispiculum* is considered a synonym of *S. trachea*.

#### INFECTION EXPERIMENTS

*Methods:* Gapeworms were removed from the trachea; the uteri dissected out, and as far as possible the gut and anterior end of the worms removed to keep the cultures clean. The eggs were placed in small dishes and incubated in a constant temperature box at a temperature ranging from 25° to 27° C. Most of the eggs were in the 4-, 8- or 16-cell stage when removed from the uterus. The cultures were kept relatively free of bacterial and fungoid growths by daily changes of water. On the 6th day the larva was clearly discernible through the eggshell. It was usually found that dissolution of the end "plugs" began on the 10th and 11th days. At the time of feeding, larvae had already hatched or could be clearly seen moving inside the shell. The species of earthworms used in the experiments were *Eisenia foetida* and *Helodrilus caliginosus*. These were kept in groups of 20 in flower pots containing soil that had been sterilized. At the end of 10 days the earthworms were washed and brushed and transferred to fresh sterilized soil before eggs were fed to them. The chicks used in the experiments were all incubator hatched and raised in the laboratory under controlled conditions.

In attempts to infect chickens directly with the infective stage of the robin gapeworm, the eggs were concentrated by drawing off as much of the water as possible. A drop of the culture was then placed on a slide and the infective eggs counted. A small quantity of flour was then added with the water until a small pellet containing the eggs was formed. This was fed to the chicks. By this method, Morgan and Clapham (1934) estimated that the size of the dose could be controlled to within an error of not more than 5 per cent. Feeding the earthworms the infective *Syngamus* eggs was accomplished by transferring the worms to dishes containing a small amount of soil, and washing infective eggs into the dishes. The earthworms remained in contact with the soil for four weeks or more before they were used in feeding experiments.

*Exper. 1:* Four one-day old chicks were fed the infective eggs and larvae of the robin gapeworm. Each chick received approximately 150 infective eggs and many recently hatched larvae. The chicks were killed one at a time at weekly intervals and examinations made of the lungs and trachea. None of the chicks revealed any indication of gapeworms.

*Exper. 2:* Each of four chickens four weeks old was fed three earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of the robin gapeworm. The chicks were killed one at a time at intervals of one week from the time of the last feeding. No evidence of gapeworms was found.

*Exper. 3:* A nest of three very young robins was kept under close observation. They were each fed one earthworm (*Eisenia foetida*) containing the larvae of the chicken gapeworm. When the robins were about two weeks old they were removed to the laboratory. Two died shortly after capture. Through an oversight one individual was not examined. The other was autopsied but no gapeworms were found. The third robin was killed and examined two weeks after it was removed from the nest. No gapeworms were found.

*Exper. 4:* A robin just learning to fly was captured and fed several earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of the robin gapeworm. Ten days after the robin was experimentally fed it was killed and examined. One small (♀ 7 mm) pair of gapeworms was recovered.

*Exper. 5:* Six one-week old chicks were fed earthworms (*Eisenia foetida*) from the soil seeded with the infective eggs of *S. trachea* from the robin. Each chick received one worm each, daily, for four days. One week later three of the chicks were killed and found uninfected. Two weeks after the chicks were experimentally fed the remaining three were killed. One chick harbored one large pair of gapeworms. No worms were found in the other two chicks.

The earthworms used in this experiment had been previously fed the infective larvae of the robin gapeworm in the laboratory. It was considered unlikely that the earthworms carried a natural infection of *Syngamus* since the gapeworm disease is rare or absent among poultry in this area. While the infection above seems to represent a cross from robin to chicken via *Eisenia foetida* it must be noted that the earthworms were collected in nature and it is not impossible that they contained the larvae of *S. trachea* from the chicken.

*Exper. 6:* In this experiment large doses of the infective eggs of the robin gapeworm were given to six one-day old chicks for a period of six consecutive days. The exact number was undetermined, but several thousand infective eggs were fed each chick. Three weeks after the first feeding, all of the chicks were destroyed and examinations were made of the lungs and trachea. Two of the chicks harbored one large pair of gapeworms each.

The number of chicks used in this experiment was small, but light infections were obtained in a third of the flock. It must be noted that the infective eggs used in this experiment differed from those of earlier

experiments since they had been in cold storage for six months before they were incubated, and increased infectivity of the eggs may have been due to this circumstance.

#### DISCUSSION

The above experiments indicate that the robin gapeworm cannot easily be transmitted to the chicken either by feeding the infective eggs, or by feeding earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of *Syngamus* from the robin. No infections in young robins resulted from feeding earthworms (*Eisenia foetida*) containing the larvae of the chicken gapeworm. An infection in a robin occurred (Exper. 4) after feeding earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of the robin gapeworm. This infection, however, might have originated before the young robin was captured. Exper. 6 seems to show definitely that robin gapeworm eggs when fed in large numbers can produce an infection with adult gapeworms in chickens. It seems doubtful if such cross-infections occur in nature.

#### SUMMARY

1. Morphologically the robin gapeworm could not be distinguished from *S. trachea* of domestic poultry. Therefore, *Syngamus tenuispiculum* Manter and Pinto, 1928, is considered to be a synonym of *Syngamus trachea*.

2. Several attempts to infect chickens with recently incubated eggs from the robin gapeworm were unsuccessful.

3. Earthworms (*Eisenia foetida*) fed incubated eggs of the robin gapeworm produced an infection in one of six chickens.

4. Eggs of the robin gapeworm kept in cold storage for six months, then incubated and fed in large numbers resulted in infections in two of six chickens.

5. Cross-infection experiments, therefore, confirm the identity of the robin and chicken gapeworms. However, because of the difficulty of cross-infection it is by no means certain that such transfers occur in nature.

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# THE INTESTINAL PHASE OF THE RESISTANCE OF RABBITS TO THE LARVAE OF *TAENIA PISIFORMIS*

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In a previous report, the senior author (Leonard, 1940) presented data to show that in the case of infections with the larvae of *T. pisiformis* in the albino rabbit, an intense and accelerated tissue reaction results in the death of most of the parasites that succeed in reaching that organ. However, in immune animals, relatively few of the onchospheres fed actually reach the liver, judging from the number of typical cysticercal lesions which may be observed there following infection. There is a possibility that the larvae are destroyed in the tissues of the liver before they have time to produce an observable lesion, but the known facts suggest the hypothesis that the intestine, or intestinal wall of immune animals becomes a serious barrier to the migration of the onchospheres from the intestine to the liver. In order to test this hypothesis, the following experiment was devised.

## EXPERIMENTAL PROCEDURE AND RESULTS

Ova of *T. pisiformis* were freed from the ripe proglottids and immersed in Tyrode's solution, to which had been added 1.0% trypsin, 0.5% dog bile by volume, and calcium carbonate to adjust the reaction to approximately pH 7.3. The ova hatched readily in this solution at 37 degrees C, and could be separated from most of the debris of the embryo-phore by gentle centrifugation. This method is similar to that used by Bullock et al (1934) in studies with *T. taeniaformis*.

Although Solomon (1934) reported that larvae of *T. pisiformis* "after being swallowed by the rabbit migrate from the stomach . . .," our findings fail to confirm this view. We have made repeated attempts to hatch the larvae in artificial gastric juice without success, even though a wide range of variations in the concentrations of both the enzyme and the acid was used. Onchospheres placed directly in the duodenum of the rabbit by operation hatched quickly, but our studies on this phase of the problem are as yet incomplete, and we are unable to state whether or not penetration occurs at the same level of the gut as hatching.

Ten normal albino rabbits were operated under ether anesthesia, and approximately 2000 artificially hatched onchospheres were injected into

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a branch of the portal vein. At the same time, five rabbits were selected from the original lot of fifteen as controls, and each was given 2000 *Taenia* ova per os. Rabbits of both groups were killed on the seventh day following this procedure, and the livers were preserved for study.

Fifteen rabbits similar in age and weight to the first group were passively immunized by the intravenous administration of 5 cc of immune serum per kilo of body weight. Twenty-four hours later, ten of these animals were operated, and each received 2000 hatched onchospheres via the mesenteric vein. The remaining five animals of this group were set aside as controls, and each was given 2000 *Taenia* ova per os. These fifteen animals were likewise sacrificed on the seventh day following infection, and livers preserved for study.

In the case of the normal animals, infection directly in the portal circulation by means of the hatched onchospheres was somewhat more successful than infection by the natural route. The number of cysticercal lesions observed on the livers of the ten operated animals varied from a minimum of 1167 lesions to a maximum of 1400 lesions, with an average number of approximately 1272 lesions per liver. In the control group which was infected by the natural route, the number of lesions varied around an average number of 956 lesions per liver, with a minimum of 788 lesions, and a maximum of 1108 lesions. The results obtained from the normal animals are summarized in Table 1.

TABLE 1.—The number of liver lesions produced in normal rabbits by inoculating into the mesenteric vein 2000 artificially hatched onchospheres of *T. pisiformis*, contrasted with controls administered 2000 *T. pisiformis* ova per os

Onchospheres via mesenteric vein		Controls; ova per os	
Animal	Number of lesions	Animal	Number of lesions
233	1230	243	1007
234	1167	244	878
235	1256	245	998
236	1301	246	1108
237	1280	247	788
238	1244	Average	956
239	1260		
240	1400		
241	1384		
242	1211		
Average	1273		

The average number of lesions per liver in the case of the immunized animals that were infected via the mesenteric vein was slightly higher than in the case of non-immune animals similarly treated, but the difference is not significant when statistical analysis is applied to the figures. In these rabbits, the number of lesions varied from a minimum of 1190 lesions to a maximum of 1460 lesions, with an average number of approximately 1339 per liver. However, in the controls on this group, which were infected per os, the number of lesions observed on the livers was greatly

reduced; the average number was only 39 lesions per liver, with a minimum of 30 lesions, and a maximum of 47 lesions. Results obtained from the immune animals are summarized in Table 2.

TABLE 2.—The number of liver lesions produced in immune rabbits by inoculating into the mesenteric vein 2000 artificially hatched onchospheres of *T. pisiformis*, contrasted with controls administered 2000 *T. pisiformis* ova per os

Onchospheres via mesenteric vein		Controls; ova per os	
Animal	Number of lesions	Animal	Number of lesions
387	1460	397	34
388	1288	398	47
389	1348	399	42
390	1401	400	30
391	1254	401	43
392	1397	Average	39
393	1305		
394	1387		
395	1190		
396	1366		
Average	1339		

#### DISCUSSION

At present, we have no data concerning the actual mechanism of the resistance factor which prevents the appearance of the great majority of the larvae in the livers of immune animals. It is not impossible, but it is improbable that the larvae are killed in the lumen of the intestine. Chandler (1935) showed that parasites in the lumen of the intestine which feed on the mucosa are not killed, but do have their nutrition interfered with so that they cannot grow and develop properly. Our studies indicate very rapid penetration of the intestine, which would make it improbable that the larvae feed until after invasion of the tissues. Therefore, the destruction of the larvae probably takes place after penetration of the mucosa, where death is due to some sort of tissue reaction. A further indication of this is the fact that Chandler (1936) showed that parenteral immunity extends to the walls of the intestine. Campbell (1936, 1938a, 1938b) showed that there are two types of immunity developed against the cysticerci of *T. taeniaformis* in rats and *T. pisiformis* in rabbits; an "early" form which brings about destruction of the parasites before encystment, and a "late" immune reaction which causes them to die after encystment. The antibodies causing "early" immunity are absorbable with fresh worm antigen and are stimulated by artificial immunization. The antibodies causing "late" immunity are not absorbable with fresh worm antigen, and do not follow artificial immunization. The "late immunity" antibodies are probably formed in response to some metabolic products of the growing worms and are comparable with the antibodies that interfere with the nutrition of *Nippostrongylus*, and which form precipitates about the mouth, anus, and excretory pore of the larvae of this worm (Sarles and Taliaferro, 1936). The destruction of the larvae

in these experiments before their encystment in the liver would seem to be due to "early immunity" antibodies described by Campbell, while the inhibition of growth and eventual death of the larvae after encystment are undoubtedly due to this author's "late immunity" antibodies.

#### SUMMARY

From the data presented, the following conclusions may be drawn:

1. In normal animals, the intestinal tract may possibly be a slight barrier to the migration of *T. pisiformis* larvae, since a greater average number of lesions was observed on the liver of animals infected via the portal circulation than in animals which were infected per os. However, this apparent natural resistance is probably not of great significance, since the loss of many ova may be accounted for by purely mechanical factors, such as intestinal peristalsis.

2. In the case of immunized rabbits, the intestinal tract is in some way a formidable barrier to the migration of *T. pisiformis* larvae, a conclusion which is supported by the significant difference between the average number of 1339 cysticercal lesions per liver in the case of animals infected via the portal circulation, and average number of 39 lesions per liver in the case of animals infected per os.

3. The resistance of immunized rabbits to infection with the larvae of *T. pisiformis* exists in two well-defined phases; an intestinal phase which is very significant, and a parenteral phase which disposes of most of the larvae that succeed in passing the intestinal barrier.

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# BLOOD PARASITES OF BIRDS OF THE DISTRICT OF COLUMBIA AND PATUXENT RESEARCH REFUGE VICINITY

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Recently a number of surveys of blood parasites of birds have been made in different parts of this country by different workers, the general purpose of which was to further the knowledge of the distribution and pathogenicity of these protozoa. This paper treats the distribution of parasites found in sampling miscellaneous birds of the District of Columbia and vicinity and contains a preliminary report on the more important phase of the problem of pathogenicity.

## METHODS

The work herein discussed was begun in the summer of 1938 incidental to a study of the cellular elements of avian blood and the data presented do not cover the entire bird population of the area. Had the bird traps been set near our laboratory in the city instead of in the suburbs, the catch would doubtless have resulted in fewer grackles and more starlings, which would probably have changed the total parasite count, since the numbers of parasites found in these two species differ so markedly. The game birds were bred on game farms in the vicinity and the ducks were taken from refuges not far away. Most of the smaller birds were trapped and released as soon as they were bled from the wing vein. A few of the birds were sick and sent in for diagnosis, and a few died in captivity.

Diagnosis was made from peripheral blood smears from live birds, except in a few cases where death of the bird made tissue smears available. The dried blood films and tissue smears were stained with Wright's stain, and examined for at least 20 minutes under oil immersion lens before they were declared negative for parasites.

Hybrid species of birds were not noted. This may account for the large number of purple grackles, some of which may have been crossed with bronze. The latter species occurs in this locality late in summer. Doubtful species of grackles were classed with the purple. More than 75 per cent of the birds were taken in the summer months.

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TABLE 1.—Distribution of parasites in hosts

HOSTS			PARASITES										
Classification and name	Common name	No. examined	No. infected	<i>Haemoproteus</i>	<i>Leucocytozoon</i>	<i>Plasmodium</i>				<i>Toxoplasma</i>	Microfilaria	<i>Trypanosoma</i>	
						Round	Long	Mixed	Undetermined				
ANSERIFORMES													
ANATIDAE													
<i>Cygnus columbianus</i> .....	Whistling swan .....	3	1	1	0	0	0	0	0	0	0	0	
<i>Branta c. canadensis</i> .....	Canada goose .....	13	1	1	0	0	0	0	0	0	0	0	
<i>Anas p. platyrhynchos</i> .....	Common mallard .....	59	21	19	1	0	0	0	0	0	1	0	
<i>Mareca americana</i> .....	Baldpate .....	1	1	0	1	0	0	0	0	0	0	0	
<i>Nettion dentata</i> .....	American pintail .....	8	4	0	4	0	0	0	0	0	0	0	
<i>Nyroca valisineria</i> .....	Canvas-back .....	1	1	0	0	0	0	0	0	0	0	0	
<i>Nyroca affinis</i> .....	Lesser scaup duck .....	3	2	0	0	0	0	0	0	0	0	0	
FALCONIFORMES													
CATHARTIDAE													
<i>Cathartes aura septentrionalis</i> ..	Turkey buzzard .....	79	18	14	2	0	0	0	0	0	9	0	
ACCIPITRIDAE													
<i>Buteo p. platypterus</i> .....	Broad-winged hawk .....	1	1	1	0	0	0	0	0	0	0	0	
<i>Buteo b. borealis</i> .....	Eastern red-tailed hawk ..	5	2	2	1	0	0	0	0	0	0	0	
GALLIFORMES													
PERDIDAE													
<i>Coturnix c. virginianus</i> .....	Eastern bob-white quail ..	93	2	1	0	0	1	0	0	0	0	0	
MELEAGRIDAE													
<i>Meleagris gallopavo</i> .....	Domestic turkey .....	4	1	1	0	0	0	0	0	0	0	0	
COLUMBIFORMES													
COLUMBIDAE													
<i>Zenaidura macroura carolinensis</i>	Mourning dove .....	5	4	3	2	0	1	0	0	0	0	0	
STRIGIFORMES													
STRIGIDAE													
<i>Otus asio naevius</i> .....	Eastern screech owl .....	7	5	1	3	0	2	0	0	0	1	0	
<i>Bubo v. virginianus</i> .....	Great horned owl .....	1	1	1	0	1	0	0	0	0	0	0	
<i>Syrinx v. varia</i> .....	Northern barred owl .....	3	2	1	2	1	1	0	0	0	0	1	
PICIFORMES													
PICIDAE													
<i>Centurus carolinus</i> .....	Red-bellied woodpecker ..	1	1	0	0	0	1	0	0	0	0	0	

TABLE 1.—(Continued)

## RESULTS

A total of 618 birds, comprising 23 families and 53 species, was examined. Of these, 188 were parasitized with from one to five types of parasites, a total of 268 infections in all birds. The parasites identified were *Leucocytozoon*, *Haemoproteus*, *Plasmodium*, *Toxoplasma*, *Trypanosoma*, and microfilaria. Table 1 shows the distribution of these parasites based on a study of the morphology in the blood films.

## DISCUSSION

No attempt was made to assign the parasites to species except in a few cases where sufficient gametocytes were found to permit average measurements and comparison with described species. For the most part, the infections were very light. The heaviest appear to have been in the brown thrashers and in a barred owl. The latter bird carried five distinct species of blood protozoa. One blue bird brought in sick from Beltsville had four species of blood parasites. Nine grackles, including one in juvenile plumage, showed triple infections, and the remaining birds had double or single infections. Of 20 young grackles, 12 were infected, nine of the 12 were infected with leucocytozoa.

One each of the following species was examined but found negative for blood parasites: Cooper hawk, red-shouldered hawk, downy woodpecker, red-headed woodpecker, yellow-breasted chat, song sparrow and meadow lark. Ten ringnecked pheasants were also negative as were five black vultures, three mocking birds, two sparrow hawks, two chimney swifts, two olive-sided fly catchers and two tufted titmice.

*Plasmodium*

Of the 53 *Plasmodium* infections herein reported, 15 were of undetermined type. In this group were placed those that showed only a few trophozoites which might have belonged to one of several species. It is not impossible that a few of them may have been *Haemoproteus* infections. Chronic cases of avian malaria, like human malaria, are sometimes impossible to detect by peripheral blood smears, especially in those species in which the parasite level is extremely low after the crisis is past, as in *P. cathemerium* and *P. circumflexum*. If a bird carries an infection for life, as is believed, chronic cases would be met with more often than acute ones in a general survey. To determine more accurately the extent of avian malaria in nature, blood inoculations should be made from the questionable bird into canaries, or better into clean birds of the same species as the host. Since this method was used in only five cases in this survey, these figures for *Plasmodium* infections represent only an estimate of the probable infection in this number of birds. One *P. elongatum* infection in a screech owl and one grackle *Plasmodium* were transmitted to canaries.

Of the 15 undetermined species of *Plasmodium*, five were in the grackles. Two of these appeared to be of similar type and different from any described species. An attempt to transmit one of these by blood and tissue inoculations resulted in an atypical infection, which is still under study in our laboratory. A total of 21 test birds—10 quail, 4 mallards, 4 pigeons, and 3 grackles—was used before a positive result could be obtained. These negative results may be due less to host specificity of the parasite than to the relative natural immunity to any *Plasmodium* of the species of birds selected for the experiment. Manwell and Herman (1935), however, also reported unsatisfactory results in estimating the *Plasmodium* infections in grackles, using a canary and an English sparrow for recipients of infected blood.

*Plasmodium elongatum* in the Bobwhite Quail (*Colinus virginianus virginianus*)

One quail not more than six months old was found to have a *Plasmodium* infection shortly after it was brought to the laboratory from nearby Maryland. Nine others brought in at the same time from the same place showed no parasites. There was no protozoan infection in the laboratory prior to this. Furthermore, it was in winter when mosquitoes are not active. The infection was discovered in December and continued for one month, after which no parasites could be found in the peripheral blood. Transmission tests to two canaries one year later, using 0.2 cc citrated blood intramuscularly gave negative results. Since birds are known to recover from this species of *Plasmodium*, this was considered to be a case of natural recovery.

The gametocytes of this quail *Plasmodium* differ slightly from the original description of *P. elongatum* Huff. Wolfson (1939) has demonstrated the gametocyte variation in a strain of *P. relictum* in the unnatural host, the duck, which variation has also been observed by the writer in a grouse *Plasmodium* when studied in quail and canaries. The inner margins of the gametocytes of this quail *Plasmodium* are often irregular, the ends sometimes appear to be square instead of round and they do not extend from one apex of the cell to the other. They do tend to bend around the host cell nucleus at one pole only, and sometimes increase the size of the host cell along the longitudinal axis. Trophozoites occupying a polar position are frequently found in immature red blood cells. These forms and adult gametocytes were fairly numerous in the blood slides, but segmenting forms were not found. These characteristics are the criteria for designating this parasite, *Plasmodium elongatum* Huff. Attempts to infect three quail with *P. elongatum* by blood inoculations from two screech owls proved unsuccessful. The injection of sporozoites from these owl infections might have given positive results, however,



since the quail herein reported must have received its infection in this manner. Dr. E. Allen (1931) reported finding *Plasmodium* in a group of quail from Georgia. "*P. praecox* is believed to be the causal organism of malaria in these birds since the gametocyte is elongate and partially surrounds the nucleus of the red cell." Dr. Allen, in a recent conversation with the writer, is of the opinion she also had a *P. elongatum* infection since she was doubtless using the nomenclature of Hartman (1927).

#### *Haemoproteus*

The *Haemoproteus* may be divided as were the *Plasmodium*, on the basis of the shape of the gametocyte, into three groups: (a) those that surround the host cell nucleus and fill the host cell cytoplasm, i.e., the *H. danielewsky* type; (b) those that grow on one side of the cell and displace the host cell nucleus, i.e., the *H. columbae* type; and (c) those that grow on one side of the host cell nucleus causing little or no hypertrophy of the cell and no displacement of the nucleus, i.e., *H. lophortyx*. Obviously this is an unsatisfactory method of classification since there is much variation between parasites of different ages, even in the same species. Mixed infections may be missed.

Of the 90 *Haemoproteus* infections found in this study, six were of the *H. danielewsky* type, while the remainder was about evenly divided between *H. columbae* type, and *H. lophortyx* type. Twenty-one of the 22 cases in grackles were of the latter type, while 16 of the 19 infections in mallards were of the *H. columbae* type, with two *H. danielewsky*, and one *H. lophortyx* type.

Three of the brown thrashers had sufficiently heavy infections to enable satisfactory measurement of the parasites and to identify them as belonging to *H. beckeri* species. Two mourning doves infected with *H. sacharovi* also had a few gametocytes that completely surround the host cell nucleus as well as a round type leucocytozoon infection which will be discussed later.

#### *Haemoproteus* of the Mallard (*Anas platyrhynchos platyrhynchos*)

*Haemoproteus* sp. has been described from the black duck by Herman (1938), who stated that it was the first report of this genus from a North American duck. The mallards from the vicinity of Washington are found to have three types of *Haemoproteus*, the most frequent of which is the *H. columbae* type. The parasites are usually numerous in the blood, and the incidence of infection varies from five out of nine parasitized in one sampling, to one in 12 infected the following summer.

The measurements of these parasites are based on the average figures from five mallards, 25 microgametes and 25 macrogametes being counted. The gametocytes are usually in contact with the host cell periphery, but

often do not touch the host cell nucleus, though it may be displaced laterally. The granules are large, black to brown, scattered in the macrogamete and polar in the microgamete.

The macrogametes stain deep blue with Wright's stain, and measure 12.6 to 16.1 microns in length and 2.6 to 4.9 microns in width, and average 14.1 by 3.7 microns. The nucleus stains pink, is usually round but sometimes triangular in shape, and is located on the periphery of the parasite nearest the host cell periphery. It varies in size from 1.4 to 4.1 microns at the widest part, and averages 2.8 microns. The granules number 8 to 26, the average being 17.6. The microgametes take very little stain, appearing hyaline to very light blue. They are 12.6 to 15.4 microns in length, and 2.8 to 5.6 microns in width, and they average 14.3 by 3.7 microns. The nucleus, when distinguishable, is a pale pink rectangle, centrally located. It averages 3.5 by 8.4 microns in size. The granules vary from 6 to 32 in number, and average 15.7 for the microgamete.

This *Haemoproteus* differs from that described from the black duck in being shorter and not usually wide enough to fill the host cell on one side. It also has a larger number of granules. It is similar in displacing the host cell nucleus.

*Haemoproteus* from the Turkey Vulture (*Cathartes aura septentrionalis*)

S. T. Darling, working in the Canal Zone, in 1912, reported finding *H. danielowsky* in a turkey buzzard *Cathartes aura*. He described it briefly but did not measure the parasite. He stated that the pigment of the microgamete had a tendency to be collected discretely in each pole. This does not read like a description of the true *H. danielowsky*, in which the gametocytes surround the host cell nucleus and fill the host cell. The description was not accompanied by illustrations. The parasite found in the turkey vulture taken near Washington, D. C., differs somewhat from Darling's description and is believed to be the first record of *Haemoproteus* from turkey vultures in the United States.

The parasites grow on one side of the host cell in close contact with the host cell periphery, not touching the host cell nucleus, but sometimes displacing it very slightly. They are frequently vacuolated. The macrogametes measure 13.3 to 15.7 microns in length and 3.1 to 4.2 microns in width; they average 14.3 by 3.8 microns. The nucleus is usually oval, centrally located, and may be either near the outer margin of the host cell or near the host cell nucleus. It averages 3 microns in size at widest part and stains pink. The macrogametes stain deep blue. The granules, varying in size from fine dots to large bacelloid structures, are scattered throughout the cytoplasm and vary in number from 8 to 38, with an

average of 27. The microgametes stain very lightly. The average measurements are 14.4 by 4.2 microns, varying from 13.3 to 16.1 microns in length and 3.3 to 4.4 microns in width. The nucleus is rarely apparent. The granules average 21 in number and are also scattered.

*Haemoproteus* from the Bobwhite Quail (*Colinus virginianus virginianus*)

*Haemoproteus* was found in the tissues of one bobwhite quail after death. Because the gametocytes of this genus of protozoa are known to round up preparatory to fertilization as soon as the blood cools, little can be said regarding morphology of this species. A number of forms which seemed little distorted were found to bear some resemblance to *H. lophortyx* of the valley quail. The host cell nucleus was displaced slightly but not to the extent of that of *H. columbae*. The parasite was usually in close contact with the host cell periphery, but seldom in contact with the host nucleus. One end was often broader than the other, the nucleus was oval, centrally located, and the scattered granules varied in number from 7 to 20. This is believed to be the first record of the genus *Haemoproteus* in bobwhite quail.

*Haemoproteus* of the Purple Grackle (*Quiscalus quiscula quiscula*)

Twenty purple grackles were found to harbor very light infections of the *H. lophortyx* type. Coatney and West (1938) have described and illustrated a *Haemoproteus* from the bronzed grackle from the vicinity of Peru, Nebraska, which they named *H. quisculus* and classed as the *H. columbae* type. This parasite in the purple grackle displaces the host cell nucleus very little, in some cases not at all; it is somewhat smaller than *H. quisculus* and exhibits little tendency to curve about the nucleus of the host cell. Since in the District of Columbia and vicinity bronzed and purple grackles, as well as hybrids, are found in the same flocks late in summer, it would not be surprising to find them carrying the same species of parasites. Five of these infections were found in birds in juvenile plumage early in the summer before the bronzed grackle had arrived. The gametocytes varied in length from 11.2 to 15.4 microns, and in width from 2.0 to 3.8 microns, and usually filled the host cell on one side. The nucleus of the macrogamete is characteristically located near one end of the parasite near the host cell periphery. The microgamete nucleus is centrally placed and diffuse. Sometimes the central vacuole described by Coatney and West for the macrogamete could be found. The granules were large, bacelloid, and varied from 6 to 12 in number. Although these linear dimensions are short compared with those given for *H. quisculus* of the bronzed grackle, the close relationship of the hosts taxonomically being considered, these parasites in the

purple grackle are sufficiently like those described by Coatney and West to call them *H. quisculus* Coatney and West.

### *Leucocytozoon*

The basis for classification of the *Leucocytozoon* is the change in the morphology wrought by the parasite on the host cell, rather than the morphology of the parasite itself. Generally birds of the large orders are parasitized by the type of *Leucocytozoon* which produces fusiform or spindle shaped host cells, while the smaller birds are infected with the round host cell type. Mixed infections are encountered in the larger orders. Of the 79 *Leucocytozoon* infections reported herein seven of them are of the fusiform type. Three of the seven infections are mixed with round forms also. One young screech owl, one barred owl, and one pintail were so infected. The long forms in the screech owl were somewhat different from those in the duck and barred owl in having a bar of host nuclear material on either side of the parasite. The *Leucocytozoon* in the purple martin was different from the other 61 round forms in passerine birds in being more oval in form and causing less distortion of the host cell, neither spindle forms nor round forms being present. Böing (1925) illustrates similar forms in the partridge (Rebhuhn) (Figs. 11, 12 and 13, Plate I) and considers them to be developmental stages in the fusiform type. Giovannola (1936) shows a similar form in a microphotograph (Fig. 12, Plate XII) from a green linnet (*Coccothraustes vulgaris*) and considers it a new species. This infection in the purple martin was very light and though search was made for some indication that it might belong to the fusiform type none was found.

### *Leucocytozoon* of the Purple Grackle (*Quiscalus quiscula quiscula*)

Forty-two of 90 grackles examined were infected with leucocytozoa, which produced more or less round gametocytes in a round host cell. The host nuclei varied in shape from a small triangular, dark-purple body, which looked like a wedge in the parasite, to a band that extended almost around the entire circumference of the parasite. In rare cases the host cytoplasm clung in patches around the nucleus and stained much paler than normal. The macrogametes stained bright blue and varied in size from round bodies about 9.8 microns in diameter to irregular ovals 12.6 by 11.2 microns. The smaller forms were more nearly spherical than the larger (possibly older) forms. The oval nucleus stained pink in varying degrees of intensity, sometimes appearing to have no stain at all. It was located at any place in the parasite, usually from the central to outer margin of the parasite away from the host cell nucleus. In less than one-fourth of the parasites a bright-red-staining karyosome could be demonstrated on the periphery of the nucleus, sometimes just outside,

sometimes just inside, its border. In some instances also there was a single vacuole near the border of the nucleus, as well as numerous vacuoles over the parasite. The microgametes averaged 12 by 8.8 microns and stained very light blue. The pale pink nucleus was often so indistinct as to make measurements impossible. In some cases it almost filled the parasite. The size was approximately 5.6 to 12 microns. In some microgametes a series of vacuoles was observed on the edge of the parasite inside the host nucleus cap. These vacuoles varied in number from 2 to 8 and were of various sizes.

*Leucocytozoon* of the Barred Owl (*Strix varia varia*)

As is shown in Table 1, this was a case of a double infection with leucocytozoa, both round and fusiform host cell types being present in small numbers. The fusiform type in the barred owl is similar to the figures of *L. ziemanni* of the little owl *Athena noctua* after Reichenow in Wenyon's Protozoology. The host cells drawn out to gently tapering ends average 40.6 microns in length, varying from 33.6 to 46.2 microns. The host cell nucleus was a thin red band curving about half of the oval parasite and varied in length from 22.4 to 25.2 microns. The microgametes average 20.3 microns in length and 5.6 microns in width, and had a large indistinct, pale central nucleus that filled a large part of the body of the light-blue parasite. The macrogamete stained deep blue, was somewhat wider than the microgamete, and had a smaller nucleus. It averaged 22.2 microns in length and 8.4 microns in width. No karyosome could be seen.

*Leucocytozoon* of the Mourning Dove (*Zenaidura macroura carolinensis*)

Two mourning doves were found with double infections of round type *Leucocytozoon* and *Haemoproteus sacharovi*. Novy and MacNeal (1905) describe the mature gametocytes of the latter as spherical, and suggest that this species of *Haemoproteus* was first observed by Sacharoff who regarded it as a *Leucocytozoon*. The round gametocytes of these mourning doves were found to lack pigment granules, and the host cell nucleus curved about the parasite, points which differentiate this from the genus *Haemoproteus*. Furthermore, one of these doves was kept under observation for one month during which time blood slides were made at intervals. The *Haemoproteus* disappeared for days at a time while gametocytes of *Leucocytozoon* could be found in varying stages of growth at almost every examination. The *Leucocytozoon* in the mourning doves appear to be the same as the species described for the purple grackle and other birds of the Order PASSERIFORMES. This is believed to be the first record of *Leucocytozoon* from North American COLUMBIDAE.



*Leucocytozoon* of the Turkey Vulture (*Cathartes aura septentrionalis*)

*Leucocytozoon* of the round host type were found in two turkey buzzards. In one bird the parasites were very scarce and showed pressure from the surrounding cells, a characteristic not observed in the passerine birds. However, the size appeared to be greater than the leucocytozoa of the smaller birds. Macrogametes from the second vulture so infected measured  $16.8\mu$  in length and  $11.2\mu$  in width, somewhat larger than similar species in the purple grackle. The cytoplasm of these parasites had the same granular structure, a karyosome could sometimes be demonstrated, and the host cell nucleus formed a cap on one side of the parasite or extended half way around it.

*Toxoplasma*

For some years workers have been observing avian species of *Toxoplasma*, rarely in the peripheral blood, more often in the internal organs, recognizable by characteristic morphology in the monocytes and lymphocytes as a single oval, blue-staining body, with a red granular nucleus, lying within a notch in the host cell nucleus. In the internal organs, where reproduction is said to take place by binary fission, a mass of dividing forms resembles schizonts of plasmodium. Because these parasites are so similar to some forms of the recently recognized exoerythrocytic stages of some of the avian malarias, they are being intensively studied at present by a number of workers, and it is hoped that their taxonomic position may be clarified thereby.

The nine cases reported in this survey were diagnosed from peripheral blood smears alone, and no dividing forms were found. *Toxoplasma* found in the purple grackles were similar to Marullaz's (1913) Fig. 1, p. 325, of *Toxoplasma avium*, to Fig. 13, plate 3, of Manwell's (1939) *Toxoplasma*, in a canary and appear to be the same as Type II *Toxoplasma* of Wolfson (1940). The parasites were rare in the blood smears, and in view of the fact that they may have some relation to other protozoa, it is of interest to note their occurrence with other parasites. Of the nine cases herein reported, all except one in the English sparrow were associated with some other type of blood parasite. A *Toxoplasma* infection occurred in a catbird that was also infected with *Haemoproteus*. Of seven grackle infections with *Toxoplasma*, two were associated with *Leucocytozoon* only, one with *Haemoproteus* only, three with *Leucocytozoon* and *Plasmodium* and one with *Leucocytozoon* and *Haemoproteus*.

*Trypanosoma*

Species of avian trypanosomes have been named on morphological grounds, although they are known to be pleomorphic, and it is not known

how many species of birds a single type of trypanosome may infect. These parasites are usually difficult to demonstrate in the peripheral blood smears, but bone marrow smears and culture methods will yield a higher incidence of infection. Packchianian (1939) states that in his culture studies of wild birds the percentage of trypanosomiasis is 80 to 82. Five infections report herein from 615 birds examined demonstrate the inferiority of dried blood films for diagnosing this infection in birds.

The trypanosomes found in two purple grackles and a bronze grackle appeared to be the same kind as *T. avium*, having myonemes, a rather wide undulating membrane, and short flagella. The average length of four forms without the flagellum was 35 microns and the width 7.3 microns. The oval pink-staining nucleus averaged 4.2 by 5.6 microns. The trypanosomes of the barred owl and the blue bird were too poorly stained to justify description. They appeared to have three or four undulations in the membrane and were without myonemes.

#### *Microfilaria*

These parasites were found in the blood stream of approximately five per cent of the birds examined. Little is known of the pathogenicity of the avian types. They have been observed in large numbers at autopsy, but what their relation to the cause of death may be is not known. Because some are periodic in appearance in the blood stream, the presence of a large number in a bird may be regarded as a periodic "shower" of these larvae. Thirty-two microfilarial infections were observed in 14 species of birds. All five species of ducks were hosts for this parasite. In five cases a careful search for the adult worm was made but none was found. *Diplotriaena* sp. are common filariae of crows, thrushes, and grackles, according to Dr. E. E. Wehr of the U. S. Bureau of Animal Industry. Filaria have also been described from "*Turdus* sp.," from ducks, from blue birds and cow birds. Further search may reveal nematodes in all these species of birds which show microfilaria.

A few characteristics of some of the forms found are recorded in Table 2. A single mallard harbored what is believed to be a double infection. The size range of the two types overlapped somewhat, but the longer forms were wavy, even curled, and were blunt at both extremities. The shorter forms were straight, pointed at the posterior, and had a large vacuole near the posterior end. The latter type, also, has been observed more commonly in mallards from other localities. A single robin found dead on the Refuge carried two sizes of microfilaria. The shorter forms were very rare in the lung and heart smears; the longer forms fairly numerous.

Purple grackles were found to harbor two types of microfilaria, the

TABLE 2.—*Microfilaria*, measurements and characteristics

Host	No. of forms measured	Length microns	Width microns	Anterior	Posterior	Sheath
Catbird .....	3	67.2 to 91 Av. 77.9	2.8 to 5.6 Av. 4.6	Blunt	Tapering	None
Crow .....	6	150 to 207.2 Av. 176.8	3 to 5.6 Av. 4.4	Blunt	Blunt to tapering	None
Grackle .....	3	133 to 154 Av. 146	4.2 to 5.6 Av. 4.9	Blunt	Blunt	None
Grackle No. 493 .....	4	122.5 to 173.3 Av. 144	4.2 to 7 Av. 5.4	Blunt	Pointed	Some had visible cross striations
Hermit thrush .....	4	126 to 182 Av. 154	4.2 to 6.3 Av. 5.2	Blunt	Tapering	None
Mallard .....	8	116.2 to 175 Av. 150.1	4.2 to 5.6 Av. 4.9	Blunt	Blunt	None. Wavy
Mallard .....	8	89.6 to 109.2 Av. 98.2	4.2 to 5.6 Av. 5.2	Blunt	Pointed	None. Straight, large vacuole near pointed end
Robin .....	6	497 to 599 Av. 520	5.6 to 9.8 Av. 7.9	Blunt	Tapering	None. Wavy. Some had cross striations
Robin .....	2	154 and 168	7.0	Blunt	Tapering	None. Straight
Screech owl .....	7	154 to 245 Av. 201.9	2.8 to 4.2 Av. 3.7	Tapering	Tapering	None. Some cross striations
Vulture .....	6	51 to 82.6 Av. 62.3	2.8 to 4.2 Av. 3.5	Blunt	Pointed	None. Some appeared to have spicule on posterior end.

more common one being unsheathed and blunt at both ends. One grackle had a few posterior pointed forms as well as some with visible cross striations.

#### SUMMARY

Blood parasites were found in 30 per cent of the 618 birds examined. A total of 268 infections, representing six genera of parasites, was found.

*Haemoproteus*, most commonly observed, was found in approximately 15 per cent of the birds examined. The following new hosts are named for this genus: Broad-winged hawk (*Buteo p. platypterus*), red-tailed hawk (*Buteo borealis borealis*), bobwhite quail (*Colinus v. virginianus*), whistling swan (*Cygnus columbianus*), Canada goose (*Branta canadensis canadensis*), and hermit thrush (*Hylocichla guttata faxoni*).

*Leucocytozoon* occurred in approximately 13 per cent of the birds examined. The following new hosts are recorded for the round type: Turkey vulture (*Cathartes aura septentrionalis*), barred owl (*Strix v. varia*), purple grackle (*Quiscalus q. quiscula*), fox sparrow (*Passerella i. iliaca*), cardinal (*Richmondia c. cardinalis*), mourning dove (*Zenaidura macroura carolinensis*), and purple martin (*Progne s. subis*).

Plasmodia were found in 8 per cent of the birds examined. These new hosts are reported: Red-bellied woodpecker (*Centurus carolinus*), fox sparrow (*Passerella i. iliaca*), and scarlet tanager (*Piranga erythromelas*).

*Toxoplasma* were found in one per cent of the birds examined, and one new host—purple grackle (*Quiscalus q. quiscula*)—was recorded.

*Trypanosoma* were found in 0.8 per cent of the birds. The purple grackle (*Quiscalus q. quiscula*) and the barred owl (*Strix v. varia*) are named as new hosts for these parasites.

*Microfilaria* were found in 5 per cent of the birds. New hosts are: Turkey vulture (*Cathartes aura septentrionalis*), screech owl (*Otus asio naevius*), catbird (*Dumetella carolinensis*), hermit thrush (*Hylocichla guttata faxoni*), and robin (*Turdus m. migratorius*).

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NEW GENERA AND SPECIES OF THE FAMILY MONORCHIIDAE (TREMATODA), WITH A DISCUSSION OF THE EXCRETORY SYSTEM

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MONORCHIIDAE were frequently encountered during a study of the TREMATODA of marine fishes of Beaufort, N. C., in the summer of 1939. Most of the species found were obviously different from any previously named, but similar to unnamed species described and figured by Linton (1905). Although it was originally planned to describe only the excretory systems of the known species of Beaufort trematodes, it seems necessary first to name four new species and two new genera of monorchiids. All specimens were first studied alive in diluted sea water and then killed in heated Gilson's fluid; later the preserved specimens were mounted in gum damar. Drawings were made of all species, both from living specimens and from whole mounts. The monorchiids studied all belong to three genera; one of these, *Genolopa*, is an old genus and the other two are described for the first time in this paper.

*Diplomonorchis* n. g.

Monorchiidae with undivided pouch-like excretory bladder; two testes, symmetrically or obliquely located on sides of body; ovary trilobate, on right side of median line, anterior to level of testes or between them; vitellaria at level of testes with some follicles extending anterior and posterior to testes; uterus coiling between testes and along sides of body anterior and posterior to testes; genital pore median ventral, between crural fork and ventral sucker; cirrus and distal part of metraterm armed with spines. Flame cell formula  $2[(2+2) + (2+2)]$ . Parasites in intestine of sea fishes.

Type species, *Diplomonorchis leiostomi* n. sp.

*Diplomonorchis leiostomi* n. sp.

(Figs. 1, 2, 3a)

With characters of the genus. Length 0.40 to 0.80 mm, average about 0.60 mm. Width 0.25 to 0.45 mm, average about 0.35 mm. Oral sucker spherical, 0.06 to 0.09 mm in diameter, average dimensions about 0.08 by 0.08 mm. Ventral sucker in middle third of body, much smaller than oral sucker, 0.04 to 0.06 mm by 0.04 to 0.06 mm. (Average 0.05 by 0.045.) Pharynx nearly spherical, about 0.04 by 0.04 mm. Esophagus usually shorter than pharynx. Intestinal crura reaching far behind testes, nearly to posterior end of body. Excretory bladder pouch-shaped, tapering to a very narrow anterior end reaching to level of ovary. Collecting tubes joining anterior end of bladder on each side, dividing at level of ventral sucker; flame cell formula  $2[(2+2) + (2+2)]$ . Testes two, symmetrical or somewhat oblique, on

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sides of body lateral to intestinal crura; level of testes about half-way between ventral sucker and posterior end. Ovary ventral and median to right intestinal cecum, partly anterior to level of testes and partly between them, divided into three distinct lobes. Vitellaria at level of testes and extending anterior and posterior to testes, dorsal to intestinal crura, consisting of a group of eight to ten follicles on each side. Uterus coiling along sides of body anterior and posterior to testes, and also filling most of intercrural space behind level of ventral sucker. Eggs thick-shelled, yellow-brown, 28 to 30  $\mu$  long and 17 to 20  $\mu$  wide in living specimens and 22 to 27  $\mu$  by 14 to 18  $\mu$  in whole mounts (See Fig. 3a). Genital pore median, just behind level of crural fork. Cirrus pouch one-fourth to one-third length of body, enclosing short nearly spherical seminal vesicle, narrow tubular pars prostatica, numerous large prostate glands, and eversible cirrus, about one-third length of cirrus pouch, armed with many short spines shaped like rose thorns about 6  $\mu$  long and 6  $\mu$  wide. Metratrerm pouch nearly as long as cirrus pouch, containing a sac-like proximal part and a distal part armed with spines which are like the cirrus spines in size and shape (See Figs. 9b, 9f).

*Host:* Spot, *Leiostomus xanthurus* Lacépède, and also occasionally in pigfish, *Orthopristis chrysopterus* (Linnaeus).

*Location:* Small intestine.

*Locality:* Beaufort, N. C.

*Type specimen:* U. S. Nat. Mus. Helm. Coll. No. 36777.

This species is represented by 136 specimens collected during the summer of 1939; it was found in 10 of the 19 spot and in 2 of the 6 pigfish examined. *Diplomonorchis* differs from *Monorcheides* (Odhner, 1905) principally in the shape of the excretory bladder (undivided instead of Y-shaped) and also in the position of the vitellaria (in the posterior half of the body instead of anterior to the ventral sucker). The only other genera of the MONORCHIIDAE with two testes are *Physochoerus* Poche, 1925, and *Paramonorcheides* Yamaguti, 1938, which like *Monorcheides* have vitellaria anterior to the ventral sucker. Manter (1940) describes the new species *Paramonorcheides bivitellosus*, remarking that "the long ceca and the posterior group of vitelline follicles of the present species might almost be of generic value." Manter's species must be transferred to *Diplomonorchis*; its name therefore becomes *Diplomonorchis bivitellosus* (Manter, 1940). *D. leiostomi* differs from *D. bivitellosus* by the smaller size of the ventral sucker (its diameter being little more than half that of the oral sucker, while in Manter's species the suckers are nearly equal), the more rounded forms of the testes, and the much larger eggs (22 to 27  $\mu$  long, compared with 19 to 20  $\mu$  in *D. bivitellosus*).

*Postmonorchis* n. g.

Monorchiidae with pouch-like undivided excretory bladder; loops of uterus mostly lateral to intestinal crura, not extending posterior to testis; testis single, in posterior part of body; vitellaria between levels of ventral sucker and testis; intestinal crura reaching nearly to posterior end of body; ovary on right side of median line; Laurer's canal and small seminal receptacle present. Cirrus and distal portion of metratrerm armed with spines; uterus opening into the spiny distal part of metratrerm pouch. Flame cell formula  $2[(2+2) + (2+2)]$ . Intestinal parasites of sea fishes. Type species, *Postmonorchis orthopristis* n. sp.

*Postmonorchis orthopristis* n. sp.

(Figs. 7, 8, 3b)

Length of unflattened whole mounts 0.35 to 0.75 mm, width 0.30 to 0.50 mm, average dimensions about 0.62 by 0.38 mm. Cuticula covered with very minute spines. Oral sucker almost spherical, much larger than ventral sucker; oral sucker about 0.10 mm in diameter, slightly wider than long; ventral sucker about 0.05 to 0.06 mm, usually nearly circular. Prepharynx shorter than pharynx. Pharynx elliptical, 0.045 to 0.050 by 0.040 to 0.045 mm. Esophagus usually shorter than pharynx. Intestinal crura reaching to posterior edge of testis, or nearly to posterior tip of body. Excretory bladder an undivided pouch reaching to level of transverse vitelline ducts. Collecting tubes entering anterior end of bladder on each side; each collecting tube dividing at level of ventral sucker into an anterior and a posterior branch, each of which, by branching again, gives off two secondary branches with 2 flame cells on each branch; flame cell formula  $2[(2+2) + (2+2)]$ . Testis single, usually in posterior third of body, median, wider than long, posterior margin with median notch. Ovary smaller than testis, margins entire or indented, on right side of median line just in front of testis. Laurer's canal and small seminal receptacle present. Vitellaria consisting of two compact groups of about 8 follicles, on each side, close to dorsal surface, just anterior to level of testis; transverse vitelline ducts joining to form vitelline reservoir in median line at level of ovary. Uterus with very compact coils almost entirely in space between intestinal crura and lateral margins of body, never entering intercrural zone behind testis; eggs with thick yellow-brown shell, 18 to 20  $\mu$  by 13 to 15  $\mu$  in live specimens and about 17 by 12  $\mu$  in whole mounts (See Fig. 3b). Genital pore median, close in front of ventral sucker. Cirrus pouch about one-third as long as body, enclosing seminal vesicle, very narrow pars prostatica, strongly developed prostate glands, and a short eversible cirrus armed with numerous narrow wedge-shaped spines (Fig. 9a) 10  $\mu$  long and about 3  $\mu$  wide. Metraterm pouch nearly as long as cirrus, containing a distal part lined with numerous slender wedge-shaped spines and a sac-like proximal part making up about three-fifths of its length. Uterus opening into spiny part of metraterm pouch either laterally or medially.

*Host*: Pigfish, *Orthopristis chrysopterus* (L.); also spot, *Leiostomus xanthurus* Lacépède.

*Location*: Intestine.

*Locality*: Beaufort, N. C.

*Syntype specimens*: U. S. Nat. Mus. Helm. Coll. No. 36778.

This species is represented by 104 specimens from 5 of the 6 pigfish examined, and 2 specimens from 2 of the 19 spot examined. *Postmonorchis* differs from *Genolopa*, *Paraprototrema*, *Prototrematoides*, *Bivesicula*, *Bivesiculoides*, *Telolecithus*, and *Asymphyiodora* in having most of the uterine folds outside of the intestinal ceca and none behind the testis; it differs from *Monorcheides*, *Physochoerus*, *Paramonorcheides*, and *Diplomonorchis* in having only a single testis; it differs from *Asymphyiodora* in having the genital pore median instead of lateral, and it differs from *Monorchis* in having an undivided excretory bladder and vitellaria posterior to the ventral sucker. The "*Mono-stomum* sp." of Linton (1905), described on p. 379 and shown in his Fig. 222, is apparently *Postmonorchis orthopristis*.

There are now 13 genera in the family MONORCHIIDAE: *Monorchis* Looss, 1902, *Monorcheides* Odhner, 1905, *Asymphyiodora* Looss, 1899, *Genolopa* Linton, 1910 (syn. *Proctotrema* Odhner, 1911), *Physochoerus*

Poche, 1925, *Telolecithus* Lloyd and Guberlet, 1932, *Bivesicula* Yamaguti, 1934, *Paraproctotrema* Yamaguti, 1934, *Proctotrematoides* Yamaguti, 1938, *Paramonorchoides* Yamaguti, 1938, *Bivesiculoides* Yamaguti, 1938, *Diplomonorchis* Hopkins, 1941, and *Postmonorchis* Hopkins, 1941. Two other genera, *Lasiotocus* Looss (in Odhner, 1911) and *Pristisomum* Looss (in Odhner, 1911), have been named but have never been described. The following key includes all the genera which have been described.

## KEY TO THE GENERA OF MONORCHIIDAE

- 1 (4) Ventral sucker and blind metraterm pouch lacking; excretory bladder V-shaped ..... 2
- 2 (3) Uterus extending posterior to testis ..... *Bivesicula*
- 3 (2) Uterus not extending posterior to testis ..... *Bivesiculoides*
- 4 (1) Ventral sucker and blind metraterm pouch present; excretory bladder undivided or Y-shaped ..... 5
- 5 (6) Genital pore on margin of body, lateral to ventral sucker ..... *Asymphyrodora*
- 6 (5) Genital pore median or nearly median, anterior to ventral sucker ..... 7
- 7 (14) Two testes present ..... 8
- 8 (13) Vitellaria in anterior half of body, lateral or anterior to ventral sucker, not extending posterior to testes ..... 9
- 9 (12) Testes near middle of body, uterus filling body behind testes ..... 10
- 10 (11) Testes nearly spherical ..... *Physochoerus*
- 11 (10) Testes longitudinally elongated, length more than twice width.   
 *Paramonorchoides*
- 12 (9) Testes in posterior third of body, uterus not extending behind testes.   
 *Monorchoides*
- 13 (8) Vitellaria mostly in posterior half of body, close to testes, with some follicles extending posterior to testes ..... *Diplomonorchis*
- 14 (7) Only one testis present ..... 15
- 15 (16) Vitellaria anterior to ventral sucker, excretory bladder Y-shaped with short stem and long furci ..... *Monorchis*
- 16 (15) Vitellaria not extending anterior to ventral sucker, excretory bladder an undivided pouch ..... 17
- 17 (18) Vitellaria behind level of testis ..... *Telolecithus*
- 18 (17) Vitellaria anterior or lateral to testis ..... 19
- 19 (20) Testis in posterior end of body, uterus not looping posterior to testis.   
 *Postmonorchis*
- 20 (19) Testis some distance from posterior end, uterus filling body behind testis 21
- 21 (22) Muscular bulb surrounding terminal part of uterus where it enters metraterm ..... *Paraproctotrema*
- 22 (21) No muscular bulb surrounding distal part of uterus ..... 23
- 23 (24) Vitellaria in longitudinally elongated, almost linear arrangement, ovary smooth-margined; a muscular spiny pouch opening into common genital atrium, separate from metraterm pouch ..... *Proctotrematoides*
- 24 (23) Vitellaria in compact, more or less rounded groups; ovary somewhat lobate in unflattened specimens; no muscular spiny atrial pouch separate from metraterm pouch ..... *Genolopa*

Two of the genera included in this key, *Bivesicula* Yamaguti, 1934 and *Bivesiculoides* Yamaguti, 1938, probably should not be placed in the family MONORCHIIDAE; they are monostomes with distinctly V-shaped excretory bladders, and completely lack both the spiny cirrus and the blind metraterm pouch characteristic of all other genera which have been placed in this family.



The question of the probable synonymy of *Proctotrema* Odhner, 1911 with *Genolopa* Linton, 1910 has been discussed by several authors, but no definite conclusion has been reached. Lloyd and Guberlet (1932) discuss this question at length, concluding that "without more detailed information concerning the morphology of the several species of *Genolopa*, especially as to the type of excretory system and the presence or absence of a seminal receptacle, any revision of the genus is, of course, impossible." Yamaguti (1934) remarks, in discussing *Proctotrema*: "This genus is probably identical with *Genolopa* . . . because it seems almost certain that Linton overlooked the true acetabulum lying behind the genital pore. If, however, Linton is right on this point, then Odhner's genus should remain valid." Manter (1940, p. 407) comments: "It is probable that the genus *Genolopa* should be restricted to monorchids with a median cluster of much longer spines in the cirrus sac, a character of *G. ampullacea*, the type species. Such a view, however, would remove most of the species now contained in the genus." In a footnote on p. 402 of the same publication, Manter says: "The removal of *Genolopa truncata* from the genus *Genolopa* leaves *Genolopa ampullacea*, the type species, still in the genus which is at least for the present recognized as distinct from *Proctotrema*."

I have examined Linton's type specimen of *Genolopa ampullacea* (U.S.N.M. Coll. No. 8525). This is a flattened specimen, well stained and quite adequate for study of all features that can be seen in a whole mount (See Fig. 4). There are two other specimens on the same slide; the larger of these is undoubtedly *G. ampullacea*, but the smaller specimen differs in several features and may possibly belong to a distinct species. The excretory bladder cannot be distinguished on any of these specimens. The type specimen agrees with Odhner's (1911) description of the genus *Proctotrema* in every point except for the more rounded shape of the oral sucker, the non-lobate form of the ovary (which may be a result of flattening, since the ovary in other species with tri-lobate ovary is more rounded in flattened specimens), and the less elongate shape of the eggs in *G. ampullacea*. Since these are minor differences, and since other species completely cover the range of differences in even these characteristics, it is obvious that *Proctotrema* Odhner, 1911 is a synonym of *Genolopa* Linton, 1910. In spite of Manter's statement, the spination of the cirrus in *G. ampullacea* is in no way exceptional, and there is no "median cluster of much longer spines in the cirrus sac" in the type specimen. *Proctotrema bacilliovatum* therefore becomes *Genolopa bacilliovatum* (Odhner, 1911), *P. lintoni* becomes *Genolopa lintoni* (Manter, 1931), *P. plectorhynchi* becomes *Genolopa plectorhynchi* (Yamaguti, 1934), *P. macrorchis* becomes *Genolopa macrorchis* (Yamaguti, 1934), *P. longicaecum* becomes *Genolopa longicaecum* (Manter, 1940), and

*P. costaricae* becomes *Genolopa costaricae* (Manter, 1940). The other species of *Genolopa* are *G. truncata* Linton, 1910, *G. trifolifer* Nicoll, 1915, *G. cacuminata* Nicoll, 1915, *G. minuta* Manter, 1931, *G. elongata* Manter, 1931, and two new species described in the present paper, a total of 14 species; *Genolopa ampullacea* Linton, 1910 is the type species.

The species of *Genolopa* studied at Beaufort in the summer of 1939 are described below.

*Genolopa lintoni* (Manter, 1931)

Forty-one specimens, from 5 of the 6 pigfish (*Orthopristis chrysopterus*) examined, were referred to this species. They varied from a length of 1.0 mm to 1.7 mm. The ventral sucker in my specimens is slightly more than half the diameter of the oral sucker, the pharynx is 0.05 to 0.07 mm long and 0.05 to 0.06 mm wide, the esophagus is nearly as long or longer than the pharynx, and the ceca extend to the posterior half of the testis or slightly beyond. Other characteristic features are shown in Figs. 10, 11, 3e and 9e, h. No seminal receptacle was seen, but it may nevertheless be present, as the arrangement of organs would make it very difficult to see this structure if present. The eggs average about 23 by 13  $\mu$ . The most striking feature of this species is the comparatively large size of the rose-thorn shaped cirrus spines (25  $\mu$  long) and the long spike-shaped spines of the metraterm (25–30  $\mu$  long); the spines of the external body covering, however, are of the same size as those in *Genolopa beauforti* (10  $\mu$  long).

Manter was evidently mistaken in saying that the "Monostomum sp." of Linton, 1905, p. 379, is a synonym of *G. lintoni*. Apparently the species referred to is the one shown in Linton's Fig. 223, for Manter quotes Linton's statement that the eggs are three times longer than wide. I have found numerous specimens of the species shown in Linton's Fig. 223, and they are very different from *G. lintoni*, as will be seen from the following description.

*Genolopa longovatum* n. sp.

(See Figs. 12, 3c, 9c)

Length usually between 0.5 and 1.0 mm but sometimes slightly exceeding these limits. Body urn-shaped, broadest in middle, tapering toward both ends but expanding again at the anterior end around the large oral sucker. Cuticular spines covering entire body. Oral sucker slightly more than twice diameter of ventral sucker (oral sucker in type 0.12 by 0.15 mm, ventral sucker 0.05 by 0.06 mm). Pharynx nearly spherical, about two-thirds diameter of ventral sucker. Prepharynx very short, seldom visible in whole mounts. Esophagus slightly longer than pharynx. Intestinal ceca ending about half-way between testis and posterior end. Single testis very close behind ventral sucker, sometimes partly dorsal to it, so that cirrus pouch is crowded forward and seldom extends posterior to ventral sucker. Cirrus and distal portion of metraterm pouch spiny; spines of cirrus wedge-shaped, nearly in form of equilateral triangle, about 8  $\mu$  long; spines of metraterm very narrow and needle-like (Fig. 9c). Ovary on right side of ventral sucker, overlapping anterior end of testis, slightly trilobate to almost spherical. Vitellaria consisting of compact groups of 8 to 10 follicles close to dorsal surface on each side of ventral sucker and anterior end of testis. Coils of uterus filling intercecal space from ovary to posterior end, slightly overlapping intestinal ceca and edges of testis. Eggs elongate, averaging 26 by 11  $\mu$ , and in living specimens often  $2\frac{1}{2}$  times as long as wide (Fig. 3c). Excretory bladder undivided, pouch-shaped.

*Host*: Pigfish (*Orthopristis chrysopterus*).

*Location*: Intestine.

*Locality*: Beaufort, N. C.

*Type specimen*: U. S. Nat. Mus. Helm. Coll. No. 36780.

The most distinctive features of this species in life are the urn-like shape, the very elongate eggs, and the almost funnel-like form of the oral sucker. The flame cells and excretory tubules were seen only once; the flame cell formula appeared to be  $2[(2+2) + (2+2)]$ , but this cannot be stated as a fact until more observations can be made. *G. longovatum* is distinguished from all other species except *G. bacilliovatum* (Odhner, 1911) and *G. costaricae* (Manter, 1940) by the elongate form of the eggs, which are however not as long as those of Odhner's species. *G. longovatum* differs from *G. bacilliovatum* in having a much greater difference in the relative sizes of the two suckers and in having a less distinctly lobed ovary, as well as in having shorter eggs ( $26\mu$  as compared with  $31\text{--}33\mu$ ) and shorter cirrus spines ( $8\mu$  compared with  $35\mu$ ). *G. longovatum* has longer ceca than *G. costaricae*, and differs also in body shape. This is almost certainly the same species as the specimen shown in Fig. 223 of Linton (1910) under the name of "*Monostomum* sp." *G. longovatum* occurred in the intestines of all of the six pigfish (*Orthopristis chrysopterus*) examined at Beaufort; a total of 54 specimens was collected.

*Genolopa beauforti* n. sp.

(See Figs. 5, 6, 3d, 9d, g)

Body elongate, length four to seven times width, covered with cuticular spines about  $10\mu$  long. Length of mature specimens 1.2 mm to 2 mm, width 0.25 to 0.35 mm. Oral sucker shaped like inverted bell with upper half twice as wide as lower half, 0.15 to 0.20 mm long and 0.13 to 0.16 mm wide; ventral sucker spherical, 0.08 to 0.11 mm in diameter, diameter about half length of oral sucker. Prepharynx longer than esophagus, nearly as long to twice length of pharynx. Pharynx very large, usually larger than ventral sucker. Esophagus short to twice as long as pharynx. Intestinal ceca reaching to posterior end of body. Genital pore at anterior edge of ventral sucker, slightly lateral to median line. A very long common genital sinus, sometimes longer than metraterm pouch, without spines. Cirrus pouch extending behind ventral sucker about half way to testis; cirrus armed with triangular spines about  $8\mu$  long, distal portion of metraterm pouch armed with slender spines about  $18\mu$  long. Uterus opening into metraterm pouch at junction of armed and unarmed portions, which is anterior to middle of pouch; loops of uterus filling all space between other organs from metraterm pouch to posterior end, but only slightly overlapping the intestinal crura. Ovary on right side just anterior to testis, nearly spherical with a slight tendency toward triangular shape. Testis about half way between ventral sucker and posterior end. Vitellaria consisting of compact groups of 8 to 10 follicles, on each side of ovary. Eggs 18 to  $20\mu$  long and 12 to  $15\mu$  wide. Excretory bladder a short undivided pouch reaching less than half way to testis; main collecting vessels dividing at level of ventral sucker into an anterior and a posterior branch; flame cell formula  $2[(2+2) + (2+2)]$ .

*Host*: Pigfish (*Orthopristis chrysopterus*).

*Location*: Intestine.

*Locality*: Beaufort, N. C.

*Type specimen*: U. S. Nat. Mus. Helm. Coll. No. 36779.

Twelve specimens of *G. beauforti* were found, in 3 of the 6 pigfish examined. This species is easily distinguished by its elongate form, long prepharynx, very large pharynx, peculiarly shaped oral sucker, and long intestinal ceca reaching to posterior end of body. *G. beauforti* is more elongate than any other species except *G. elongata* (Manter, 1931) and *G. cacuminata* Nicoll, 1915 both of which have ventral suckers larger than oral suckers. The pharynx of *G. beauforti* is larger (in relation to the sizes of the suckers) than in any other species.

#### THE EXCRETORY SYSTEM OF MONORCHIIDAE

The excretory systems of four species, in four different genera, are now completely known. These are *Diplomonorchis leiostomi* Hopkins, 1941, *Postmonorchis orthopristis* Hopkins, 1941, *Genolopa beauforti* Hopkins, 1941, and *Monorcheides cumingiae* (Martin, 1938). The life cycle of the last species is the only one known in this family. Martin (1939) found that *Cercaria cumingiae* Martin, 1938, a cercaria with eyespots but without stylet and with a fairly long undivided tail bearing peculiar lateral lappets instead of spines or setae, encysts in the clam which serves as first intermediate host, and becomes adult in the anterior intestine of eels and flounders. It is interesting to note that the excretory bladder of *Monorcheides cumingiae* (at least in the cercaria and metacercaria stages) has an undivided bladder like that of *Genolopa*, a fact which casts doubt on Odhner's statement that *Monorcheides* has a Y-shaped bladder. All of the known excretory systems have the flame cell formula  $2[(2+2) + (2+2)]$ , which we may assume with some confidence to be the formula for all members of the family MONORCHIIDAE.

Members of several other families have the flame cell formula  $2[(2+2) + (2+2)]$ . In the MICROPHALLIDAE, species of the genera *Microphallus*, *Cornucopula*, *Spelotrema*, *Levinseniella*, and *Maritrema* have been shown by various authors to have this formula. In the HETEROPHYIDAE, *Caecicola*, according to Lundahl (1939) and *Centrocestus*, according to Yamaguti (1938a) have the  $2[(2+2) + (2+2)]$  formula. MacFarlane (1939) reports this formula for *Coitocaecum*, a member of the COITOCAECIDAE or OPECOELIIDAE. Dobrovolny (1939) found the same formula in *Plagioporus* of the family ALLOCREADIIDAE, and I have found it in the allocreadiid-like genera *Helicometra* and *Cymbephallus* (unpublished observations). *Tergestia*, in the STERNGOPHORIDAE (= FELLODISTOMIDAE) also has the  $2[(2+2) + (2+2)]$  formula (Hopkins, 1940). *Zoogonus*, in the ZOOGONIDAE, has been reported to have this formula by Stunkard (1938).

Considering the marked differences in their adult structures and life cycles, it seems unlikely that the possession of the same flame cell formula indicates close genetic relationships between all of these families. It

would be more plausible, perhaps, to suggest that the  $2[(2+2) + (2+2)]$  formula represents a primitive condition usually passed over early in the embryonic development of cercariae, but that in many possibly unrelated genera the development of the excretory system has been retarded so that it remains in the primitive condition. On the other hand, if genera which had been placed in the same family were found to have widely different flame cell formulae, it would certainly cast doubt on the closeness of their relationship, especially if the difference were found in the cercaria as well as in the adult stages.

## SUMMARY

1. The new genera *Diplomonorchis* and *Postmonorchis* are described.
2. The new species *Diplomonorchis leiostomi* (from *Leiostomus xanthurus*), *Postmonorchis orthopristis*, (from *Orthopristis chrysopterus*), *Genolopa longovatum* (from *Orthopristis chrysopterus*), and *Genolopa beauforti* (from *Orthopristis chrysopterus*) are described, all from Beaufort, N. C. Type specimens of all new species have been deposited in the U. S. National Museum.
3. *Proctotrema* Odhner, 1911 is declared a synonym of *Genolopa* Linton, 1910.
4. The excretory systems of *Diplomonorchis leiostomi*, *Postmonorchis orthopristis*, and *Genolopa beauforti* are described, all with undivided excretory bladder and the flame cell formula  $2[(2+2) + (2+2)]$ .
5. Keys are presented for identification of the genera of the MONORCHIIDAE.
6. The possession of the  $2[(2+2) + (2+2)]$  flame cell formula by genera in several other families is discussed.

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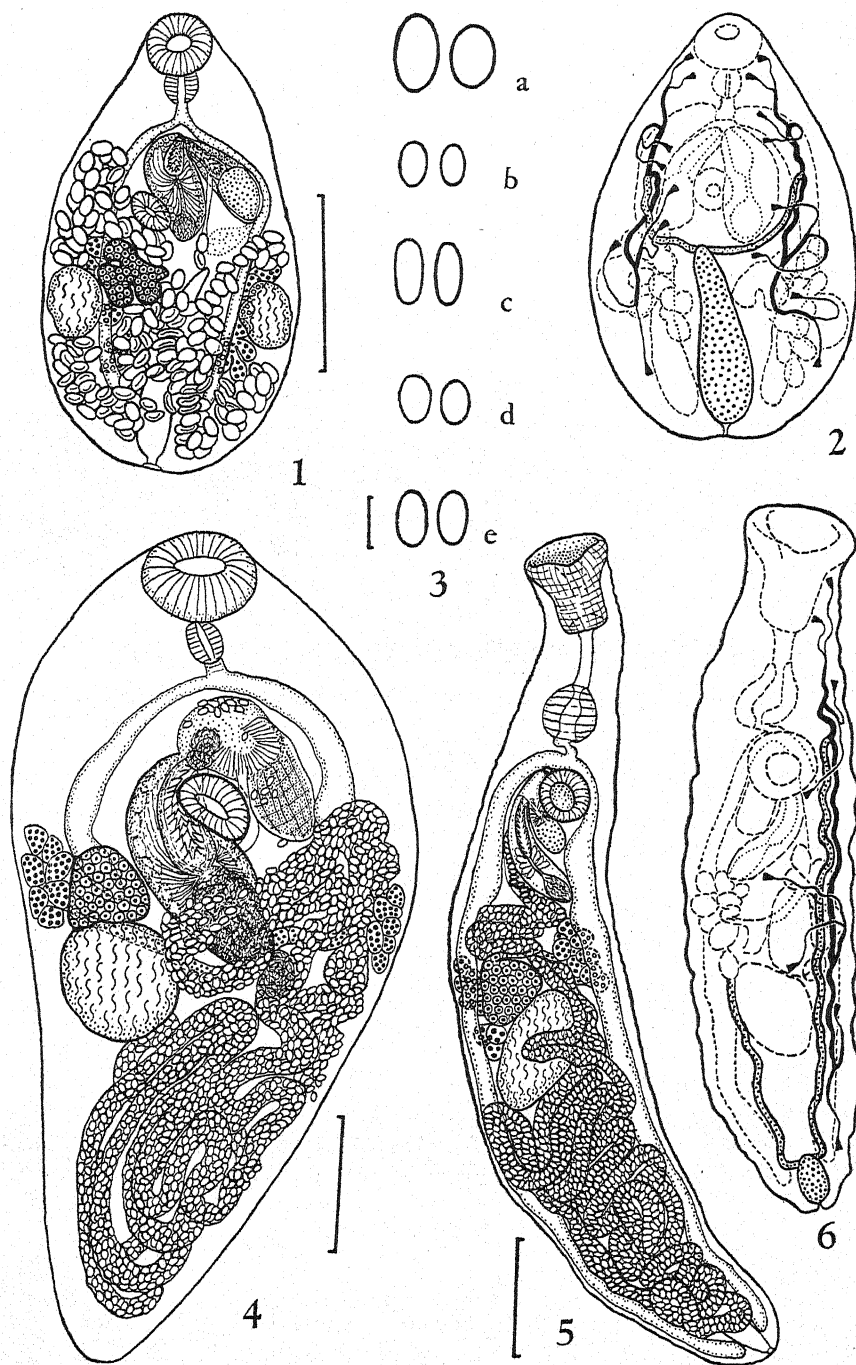


PLATE I

## EXPLANATION OF PLATES

## PLATE I

FIG. 1. *Diplomonorchis leiostomi* n. sp., ventral view of whole mount. Scale line=0.2 mm.

FIG. 2. *Diplomonorchis leiostomi*, camera lucida drawing of living specimen, dorsal view. Excretory tubules and flame cells drawn freehand.

FIG. 3. Camera lucida drawings of typical eggs of the 5 species described in this paper. The eggs on the left are drawn from living specimens, those on the right from whole mounts in gum damar. Scale line=0.02 mm.

- a, *Diplomonorchis leiostomi*
- b, *Postmonorchis orthopristis*
- c, *Genolopa longozatum*
- d, *Genolopa beauforti*
- e, *Genolopa lintoni*

FIG. 4. *Genolopa ampullacea* Linton 1910, type specimen, drawn with camera lucida, ventral view. Scale line=0.2 mm.

FIG. 5. *Genolopa beauforti* n. sp., camera lucida drawing of whole mount, ventral view. Scale line=0.2 mm.

FIG. 6. *Genolopa beauforti*, camera lucida drawing of living specimen, with excretory tubules and flame cells added freehand.

## PLATE II

FIG. 7. *Postmonorchis orthopristis* n. sp., camera lucida drawing of living specimen, with excretory tubules and flame cells added freehand.

FIG. 8. *Postmonorchis orthopristis*, camera lucida drawing of whole mount, ventral view. Scale line=0.2 mm.

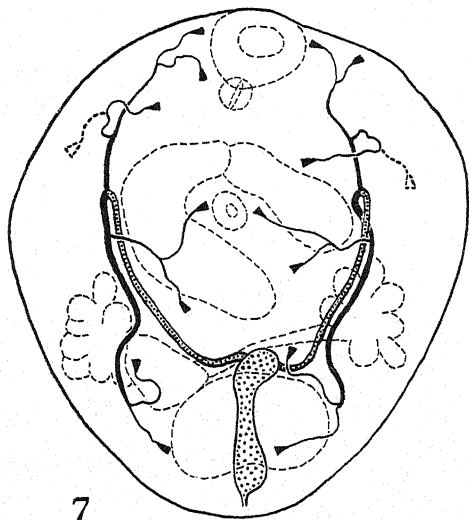
FIG. 9. Genital spines of the 5 species described in this paper; camera lucida drawings from living specimens. Scale line=0.02 mm.

- a, cirrus spines of *Postmonorchis orthopristis*
- b, cirrus spines of *Diplomonorchis leiostomi*
- c, cirrus spines of *Genolopa longozatum*
- d, cirrus spines of *Genolopa beauforti*
- e, cirrus spines of *Genolopa lintoni*
- f, metraterm spines of *Diplomonorchis leiostomi*
- g, metraterm spines of *Genolopa beauforti*
- h, metraterm spines of *Genolopa lintoni*

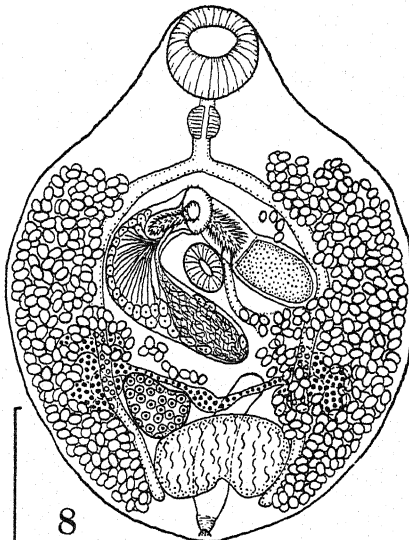
FIG. 10. *Genolopa lintoni* (Manter, 1931), camera lucida drawing of whole mount, ventral view, with eggs omitted for the sake of clearness. Scale line=0.2 mm.

FIG. 11. *Genolopa lintoni*, camera lucida drawing of whole mount, dorsal view. Scale line=0.2 mm.

FIG. 12. *Genolopa longozatum* n. sp., camera lucida drawing of whole mount, ventral view. Scale line=0.2 mm.

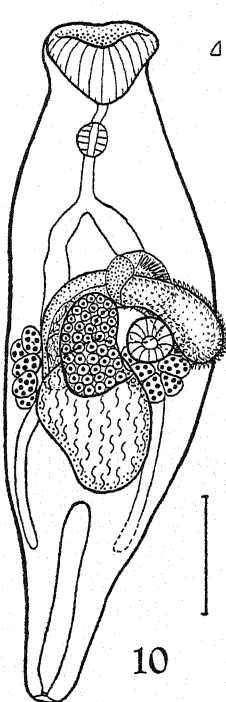


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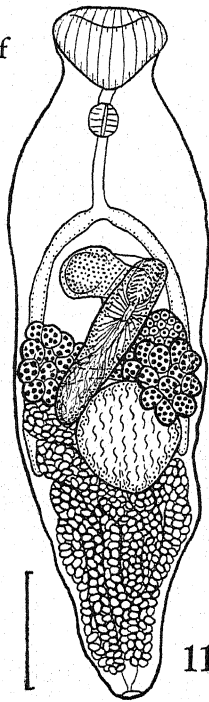


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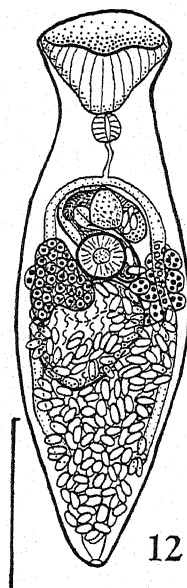


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# ON DISTRIBUTION RELATIONSHIPS BETWEEN CALIFORNIA TIDE POOL FISHES AND THEIR MYXOSPORIDIAN (PROTOZOAN) PARASITES

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Workers in the field of taxonomic parasitology are often impressed with the lack of correlation between the distribution of parasites and the distribution of their hosts. Some species of parasites are narrowly confined to a particular host species in one locality, while others have a distribution as wide as the earth's surface and involve many different species of hosts. The writer has become interested in variation in host-parasite relationships through investigations of myxosporidian parasites of tide pool fishes.

A description of a new species of myxosporidia will precede a discussion of distribution.

*Sphaeromyxa lateralis* n. sp.

(Figs. 1-3)

*Trophozoite* leaf-like, flattened. Length up to 1 and  $\frac{1}{2}$  millimeters. Periphery only slightly differentiated from endoplasm. Each pansporoblast develops two spores. Polysporous. Small ameboid stages occasionally present which have clear needle-like pseudopodia (Fig. 1), and measure 15 microns in total length. No movement observed.

*Spore* 26 microns long (suture diameter) by 8 microns wide; oval, slightly curved in side view. Polar capsules 6.3 by 8.6 microns. Polar filaments loosely coiled, approximately 22 microns long. The capsulogenous nucleus of one end usually takes a much darker stain (iron hematoxylin) than that of the other end of the spore. Sporoplasm lightly granular, with two large nuclei. Each nucleus with a single karyosome. Suture line indistinct. Figs. 2 and 3.

*Host*: *Artedius lateralis* Giard.

*Location*: Gall bladder.

*Locality*: Tide pools of Santa Barbara, California.

In comparison with the other species of the genus, *Sphaeromyxa lateralis* is most similar to *S. gibbonsia* Noble from the gall bladder of *Gibbonsia elegans elegans* and *G. metzi*. The principal difference is in the shape of the polar capsule which is markedly shorter and wider in *S. lateralis*. Only three infected hosts have been found. These were collected during November and December, 1939.

The gall bladders of *Dialarchus snyderi* Greeley from Santa Barbara (October 1937), Point Conception (November 1939 and August 1940) and from Santa Rosa Island (August 1940) were found to be heavily

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infected with *Myxidium incurvatum* Thélohan. This species has been reported from the Mediterranean Sea, the North Sea and from the American Atlantic and Pacific coasts. The characters of the parasite found in *Dialarchus snyderi* are as follows:

Trophozoite irregularly globular, with rounded, blunt pseudopodia (Fig. 4). Very slow ameboid movement. Pseudopodia slightly less granular than cytoplasm of body. Little distinction between ectoplasm and endoplasm. Latter with large refractive granules. Mature size 30 microns in diameter. Disporous.

Spore (Figs. 5 and 6) averages 4.5 by 11 microns; polar capsules average 1.8 by 3.8 microns. Spindle shape in front view, with very pointed ends; may be swelled in the middle. In side view the whole spore is twisted into an S-shape. The axes through the long diameters of the polar capsules are not parallel. Shell wall thin and smooth. Sporoplasm lightly granular and fills spore, with two nuclei which are indistinct in living spores. Suture line faint. Filaments not observed.

In his original description Thélohan (1892) described a disporous condition for *Myxidium incurvatum* from *Nerophis aequoreus*, *N. annulatus*, etc., from the southern coast of France, while Jameson (1929) described a monosporous condition for the same parasite in *Sebastodes caurinus* Richardson from Pacific Grove, California. The forms described above from *Dialarchus snyderi* are smaller than those described by Thélohan.

In the gall bladder of one specimen of *Gibbonsia elegans elegans* (Cooper) collected at Point Conception (October 1939) a heavy infection of *Leptotheca sphaerula* was found.

*Gibbonsia metzi* Hubbs was found to be the host of two other myxosporidia. In three of these fish from Point Conception (November 1939) the gall bladders were found to be infected with *Ceratomyxa blennius*. The only other reported infection of California tide pool fishes by this genus was by Jameson (1929). He described *C. gracilis* in *Gibbonsia metzi* (evides) from Pacific Grove. In the gall bladder of one specimen of *G. metzi* from Santa Barbara (April 1940) the writer found an abundance of *Trilospora californica* with a small number of *Leptotheca elegans*.

In the gall bladders of two specimens of the blind goby, *Typhlogobius californiensis*, from Santa Barbara (April 1940) were found moderate infections of *Leptotheca elegans*. These parasites were mixed with *Trilospora californica*.

*Leptotheca elegans* has also been found in the gall bladder of *Artedius lateralis* from Santa Barbara (December 1939) together with *Sphaeromyxa lateralis* described above. The former differed from the original description (Noble 1938) in that no movement of the trophozoite was observed, and the spores averaged 7 by 22 microns instead of 9 by 17 microns.

A list of tide pool fishes of California from which myxosporidia have

been described, together with their parasites, was published in a previous paper (Noble 1939). Herewith is the same list with the additions mentioned above.

## HOST LIST WITH PARASITES

- Arbaciosus rhessodon*  
*Sphaeromyxa ovula* in gall bladder  
*Artedius lateralis*  
*Sphaeromyxa lateralis* in gall bladder  
*Leptothea elegans* in gall bladder  
*Auchenopterus intergripinnis*  
*Leptothea elegans* in gall bladder  
*Clinocottus analis*  
*Ceratomyxa gracilis* in gall bladder  
*Dialarchus snyderi*  
*Myxidium incurvatum* in gall bladder  
*Gibbonsia elegans*  
*Leptothea elegans* in gall bladder  
*Sphaeromyxa gibbonsia* in gall bladder  
*Trilospora californica* in gall bladder  
*Leptothea sphaerula* in gall bladder  
*Ceratomyxa blennius* in gall bladder  
*Gibbonsia metzi*  
*Leptothea sphaerula* in urinary bladder  
*Leptothea elegans* in gall bladder  
*Sphaeromyxa gibbonsia* in gall bladder  
*Ceratomyxa gracilis* in gall bladder  
*Ceratomyxa blennius* in gall bladder  
*Trilospora californica* in gall bladder  
*Hypsoblennius gilberti*  
*Ceratomyxa blennius* in gall bladder  
*Rimicola eigenmanni*  
*Leptothea compressa* in urinary bladder  
*Typhlogobius californiensis*  
*Trilospora californica* in gall bladder  
*Leptothea elegans* in gall bladder

The following list illustrates differences in infection range of myxosporidia among California tide pool fishes:

- |                                     |                                    |
|-------------------------------------|------------------------------------|
| <i>Ceratomyxa blennius</i>          | <i>Leptothea sphaerula</i>         |
| <i>Gibbonsia elegans</i>            | <i>Gibbonsia elegans</i>           |
| <i>Hypsoblennius gilberti</i>       | <i>Gibbonsia metzi</i>             |
| <i>Gibbonsia metzi</i>              | <i>Myxidium incurvatum</i>         |
| <i>Ceratomyxa gracilis</i>          | <i>Dialarchus snyderi</i>          |
| <i>Gibbonsia metzi</i>              | <i>Sphaeromyxa gibbonsia</i>       |
| <i>Ceratomyxa obesa</i>             | <i>Gibbonsia elegans</i>           |
| <i>Clinocottus analis</i>           | <i>Gibbonsia metzi</i>             |
| <i>Leptothea compressa</i>          | <i>Sphaeromyxa lateralis</i>       |
| <i>Rimicola eigenmanni</i>          | <i>Artedius lateralis</i>          |
| <i>Leptothea elegans</i>            | <i>Sphaeromyxa ovula</i>           |
| <i>Artedius lateralis</i>           | <i>Arbaciosus rhessodon</i>        |
| <i>Auchenopterus intergripinnis</i> | <i>Trilospora californica</i>      |
| <i>Gibbonsia elegans</i>            | <i>Gibbonsia elegans</i>           |
| <i>Gibbonsia metzi</i>              | <i>Gibbonsia metzi</i>             |
| <i>Typhlogobius californiensis</i>  | <i>Typhlogobius californiensis</i> |

Of particular interest, from the standpoint of distribution, is the presence of *Myxidium incurvatum* which has been found in only one species of fish in this locality, but which has been reported from at least sixteen different species of fish, including both fresh water and marine forms, from various parts of the world.

The littoral flora and fauna of the California Coast north of Point Conception is unlike the littoral flora and fauna south of Point Conception. This is due to a difference in temperature and direction of water and air currents in these two regions. Several species of fishes are common to both localities. From the point of view of host-parasite relationships it is of interest to know whether the distribution of the parasites of these fishes parallels that of their hosts.

For this study the gall bladders of tide pool fishes were examined, and their infection by myxosporidian parasites noted.

In comparing the myxosporidia of fishes occurring north of Point Conception with those occurring south of this Point, Jameson (1931) states that there is "marked similarity between the two areas." The writer has found, on the basis of the study of tide pool fishes alone, that a similarity is not very marked.

The gall bladders of approximately 125 specimens of *Clinocottus analis* from Point Conception southward have been examined by the writer, and no parasites have been found. These fishes were collected over a period of three years, and during all seasons of the year. Jameson (1929) described *Ceratomyxa obesa* from *Clinocottus analis* collected in Monterey Bay, approximately 175 miles north of Point Conception. The absence of parasites in the fishes from southern waters suggests at least a physiological difference between the two groups of hosts. The writer recently (August) collected 9 specimens of this fish from Monterey Bay, and was unable to find myxosporidia in any of them. There is evidence from the work of Bolin (unpublished) that a subspecific separation of the two groups of fish is unwarranted.

*Dialarchus snyderi* Greeley is abundant in tide pools along the entire coast of California. The writer has collected these fishes during all seasons of the year on both sides of Point Conception from Monterey Bay southward to Ventura. Infection of the gall bladders of *D. snyderi* with *Myxidium incurvatum* Thélohan is equally common in all localities studied. This condition is to be expected because of the world wide distribution of *M. incurvatum*.

*Gibbonsia elegans* (Cooper) and *Gibbonsia metzi* Hubbs are both abundant along the coast of California, and both harbor, with one exception, the same species of myxosporidian parasites. Here is an example of two distinct species of hosts, found under varying environmental conditions, which are both subject to infection by essentially the same gall

bladder parasites. From a study of the parasites of this genus the writer concludes that the hosts are physiologically more similar than is indicated by their structural characteristics.

Of the fishes extensively studied by the writer, *Montereya recalva* (Greeley) is the only common tide pool fish of central and southern California which is free from gall bladder parasites. Approximately 100 gall bladders were examined, taken throughout the year from Point Conception and the Channel Islands, and all were found to be negative for myxosporidia.

When we consider the bottom feeding habits of the tide pool fishes, with the abundance of algae and small crustaceans usually present, we find a combination of conditions particularly favorable to the development and transmission of the myxosporidia. Bangham and Hunter (1939) made a similar observation in their studies of fish parasites of Lake Erie. They found that the fish at the western end of the lake, which is shallower and weedier, supported greater numbers and greater variety of parasites when a fish was infected. Davis (1917) points out that "it is very common to find two or more species of myxosporidia living in the gall bladder or the urinary bladder at the same time."

Somewhat opposed to the above findings are those of Cross (1938) who concluded that "fish that harbor large numbers of parasites of one kind have a tendency toward a light infection, or none, with other parasites." A glance at the host list on page 411 shows that out of ten different species of tide pool fishes only two harbor more than two species of myxosporidian parasites. Even in the genus *Gibbonsia*, which harbors 6 different species of myxosporidian parasites, the writer usually found only one species of myxosporidia present in any one fish, and never more than two of these sporozoans, although occasionally other parasites (worms) were encountered. Thus the study of myxosporidia of tide pool fishes supports the conclusions of Cross.

The ability of a parasite to live normally in many different species of hosts indicates a pronounced adaptability on the part of both host and parasite. This adaptability could be explained on the basis of a generalized condition of the parasite. According to this interpretation the parasite has not progressed far enough, phylogenetically speaking, to attain the specialization which permits it to dwell in only one particular species of host. When we compare *Leptotheca elegans*, however, which is found in five different species of host, with *Leptotheca compressa*, which is confined to *Rimicola eigenmanni*, we can observe no differences which would indicate that the latter is more specialized. Since *L. elegans* has not been found in *Rimicola eigenmanni* we can assume that this fish and parasite are physiologically incompatible. If a difference in generalization of the above mentioned parasites exists it must be of a physiological nature.



The physiology of the myxosporidia has merited little attention on the part of investigators, and offers an open field for research.

## SUMMARY

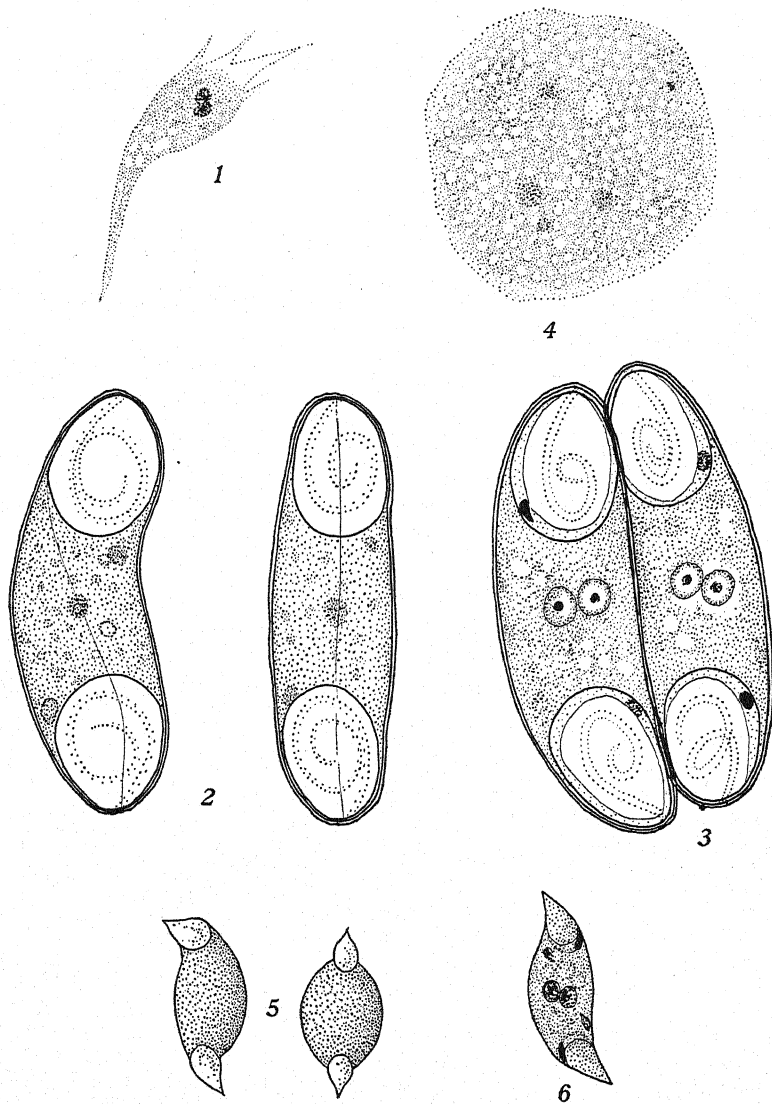
*Sphaeromyxa lateralis* n. sp. from the gall bladder of *Artedius lateralis* has been described. A revised list of tide pool fishes of California with their myxosporidian parasites, and a list of myxosporidian parasites of California with their hosts is included.

Investigations on the host-parasite problem with relation to distribution of tide pool fishes and their myxosporidian parasites result in the following conclusions:

1. Two groups of hosts, both belonging to the same species but separated each into a different environment, may sometimes be distinguished by differences in their parasitic fauna.
2. Some parasitic species are very resistant to change, and are able to maintain their diagnostic characteristics in several species of hosts in different environments.
3. There is often no close correlation between the structural similarities of two species of hosts and the similarities of the parasites with which they are infected.
4. Fishes living in the littoral zone, where there is an abundance of plant life, are generally infected with a greater variety of parasites than are pelagic fishes.
5. The presence of large numbers of parasites of one species in a fish usually excludes the presence of large numbers of other species of parasites in that fish.

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## EXPLANATION OF PLATE

All figures drawn with the aid of a camera lucida. Magnification  $\times 2230$ .

Fig. 1. Living trophozoite of *Sphaeromyxa lateralis*.

Fig. 2. Two living spores of *Sphaeromyxa lateralis*.

Fig. 3. Pair of stained spores of *Sphaeromyxa lateralis*. (Brazil's Bouin's, iron hematoxylin.)

Fig. 4. Young living trophozoite of *Myxidium incurvatum*.

Fig. 5. Two living spores of *Myxidium incurvatum*.

Fig. 6. Stained spore of *Myxidium incurvatum*. (Brazil's Bouin's, iron hematoxylin.)



COMPARATIVE EFFICIENCY OF ZINC SULFATE AND  
SUGAR SOLUTIONS FOR THE SIMULTANEOUS  
FLOTATION OF COCCIDIAL OÖCYSTS  
AND HELMINTH EGGS

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A number of substances has been recommended for use in detecting protozoan cysts and helminth eggs in feces and soil. For many years, it has been known that certain of these substances were more efficient in floating protozoan cysts while others were more satisfactory in recovering helminth eggs. Until recently, however, little or no attempt was made to collect experimental data on the efficiency of substances for the simultaneous flotation of both protozoan oöcysts and helminth eggs from the same medium. Faust et al (1938, 1939) tested several techniques for the recovery of protozoan cysts and helminth eggs simultaneously from human feces and found that zinc sulfate solution having a specific gravity of 1.180 recovered the largest number of these forms. Garcia and Pesigan (1940) demonstrated that the IHP flotation method (named after Institute of Hygiene, Department of Parasitology, University of Philippines) involving the use of cupric nitrate having a specific gravity of 1.180 was just as effective in the diagnosis of helminth ova and protozoan cysts in human feces as zinc sulfate technique. These authors recommended the cupric nitrate technique for the following reasons: (1) smear preparations do not dry, even without the cover slip, for one month or more; (2) cupric nitrate preparations give a clearer and cooler visual microscopic field thus alleviating eye strain; (3) the fecal material is stained bluish-green while the eggs and cysts remain unstained; and (4) the high solubility of cupric nitrate gives a wider range of specific gravities than zinc sulfate. Pesigan (1940) tested both the zinc sulfate and cupric nitrate techniques for the simultaneous flotation of helminth eggs and protozoan cysts from human feces and found that each gave an efficacy of approximately 90 per cent.

The present paper presents data on the comparative efficiency of zinc sulfate and sugar solutions for the simultaneous flotation of coccidial oöcysts and helminth eggs from naturally infected and artificially inoculated chicken feces and soil.

MATERIALS AND METHODS

Tests were conducted on five fecal samples and five soil samples, the latter of a sandy loam nature. Three of the soil samples and two of the

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fecal samples were inoculated with known numbers of coccidial oöcysts and nematode eggs while the two remaining soil samples and the three remaining fecal samples, which were collected from infected poultry yards and infected poultry, respectively, contained unknown numbers of oöcysts and eggs.

Of the 3 inoculated soil samples, the first and second (Nos. 1 and 2 on chart) were collected from an area previously unused as a chicken yard; they were thoroughly dried in an oven at 50° to 60° C before being inoculated. An analysis<sup>1</sup> of a small portion of one of these dried samples showed that it contained 0.04 per cent nitrogen, 2.15 per cent organic matter, 16 per cent moisture, and had a pH of 4.2. A 125 cc portion of sample 1 was made into a paste by adding 25 cc of water; this reduced the volume of the dried soil to 74 cc. To this paste were added 193,750 oöcysts and 19,000 nematode eggs. A 109 cc portion of sample 2 was prepared in a manner similar to sample 1 except that 49,500 oöcysts and 22,400 nematode eggs were added.

The third inoculated soil sample (No. 3 in chart) was collected from the surface of the ground around the feeders and shelters of an infected poultry yard. An analysis of a portion of this latter sample, which had previously been comminuted and dried in an oven at a temperature of 50° to 60° C for more than a month, showed that it contained 0.27 per cent nitrogen, 4.16 per cent organic matter, 34 per cent moisture, and had a pH of 7.76. A preinoculation examination of several samples of this soil showed that an average of 14 oöcysts per 2 cc of soil were present. A 125 cc portion of this sample was mixed thoroughly with 63 cc of tap water; this increased the volume of the dried soil to 136 cc. To this paste there were added 121,000 oöcysts and 37,500 nematode eggs.

The fourth and fifth soil samples (Nos. 4 and 5) were scraped from the surface of the ground around the feeders and shelters of infected poultry yards. A small portion of each sample was comminuted and screened. The screenings from soil sample No. 4 were thoroughly mixed with water before being examined for eggs and oöcysts. Soil sample No. 5 was dried in an oven at a temperature of 50° to 60° C for a month and then inoculated with an unknown number of eggs and oöcysts before being tested.

Fecal samples (Nos. 6 and 7) were collected from parasite-free chickens. Ninety cc of sample No. 6 were inoculated with 413,200 oöcysts and 90,000 eggs. The volume of the inoculated sample was 96 cc. Sample No. 7 was dried in an oven at 50° to 60° C for one month, then removed and ground into a powder with a mortar and pestle. One hundred and twenty-five cc of the ground droppings were mixed into a smooth paste

<sup>1</sup> Analysis made by A. Yelmgren of the Division of Soil Chemistry and Physics, Bureau of Plant Industry.



with 150 cc of water; the volume of this mixture was 175 cc. To 135 cc of this paste there were added 121,000 oöcysts and 37,260 eggs, increasing the volume of the mixture to 170 cc.

Fecal samples (Nos. 8, 9, and 10) were collected from chickens naturally infected with coccidia and helminths. These chickens were confined in cages for a week and fed a mash free of grit before the samples were taken.

The prepared soil and fecal samples were placed in sealed jars which were kept in a refrigerator until ready for use.

Suspensions of sporulated oöcysts used to inoculate the fecal and soil samples were obtained by culturing the unsporulated oöcysts of *Eimeria maxima*, *E. mitis*, *E. acervulina*, and *E. tenella* in 2.5 per cent potassium dichromate solution for two to three days. On completion of sporulation, the oöcysts were washed free of potassium dichromate with tap water and then strained through cheesecloth and cotton to remove all debris. The number of oöcysts in each suspension was then determined from counts made with the aid of a Fuchs-Rosenthal blood counting chamber 0.2 mm deep. A pipette was used to stir the suspension of oöcysts to distribute them equally throughout the liquid before making each count. Counts on individual samples were continued until the numbers of oöcysts of two or more counts were identical. At least five and usually more counts were made of each suspension and then averaged. This average was then used to estimate the total number of oöcysts desired to be used in the inoculum.

The suspensions of helminth eggs were prepared by culturing the freshly collected eggs of the worms *Ascaridia lineata* and *Heterakis gallinae* in a 2 per cent solution of potassium dichromate for about two weeks. The egg suspension was then strained through cheesecloth to remove the debris and then washed free of potassium dichromate with tap water. Several counts were made of each suspension by the Stoll dilution method and the average of these used to determine the number of eggs desired to be used in the inoculum.

The inoculum was prepared by mixing thoroughly definite amounts of the egg and oöcyst suspensions. After inoculation, the soil or fecal sample was mixed thoroughly with a spatula to insure a uniform distribution of eggs and oöcysts.

The method adopted for the flotation of helminth eggs and protozoan cysts from fecal and soil samples was as follows: A 2 cc subsample, which was obtained by means of a U. S. Bureau of Plant Industry watch glass (27 mm in diameter and 8 mm high), was placed in a 125 cc Erlenmeyer flask containing 33 cc of tap water and glass beads. The contents of the flask were shaken vigorously for 5 minutes and then strained through 4 thicknesses of washed cheesecloth into two 15 cc centrifuge tubes. The

tubes were centrifuged at 1,050 RPM for 3 minutes. The supernatant liquid was poured off and a small amount of the flotation medium added to each tube. The contents of one tube were mixed thoroughly with an applicator stick and poured into the other. Enough of the flotation medium was then added to the latter tube to bring the level of the suspension even with the rim of the tube. A clean No. 2 coverslip was placed across the rim of the tube, care being taken to see that no air bubble or at most not more than a very small one was present underneath the slip. The tube was centrifuged at 1,050 RPM for 3 minutes. The coverslip was then removed to a slide for enumeration of eggs and oöcysts present thereon. This same procedure was carried out a second, sometimes a third or even a fourth time for the purpose of determining the total number of eggs and oöcysts recoverable from the subsample. Table I shows in tabulated form the results obtained from the examination of two coverslips of each sample tested as described above.

The zinc sulfate solution (sp gr 1.200), which was used in the present tests, was prepared by placing 331.4 grams of the chemical in a 1,000 cc glass cylinder and then adding enough water to bring the level of the solution to the one liter mark. The sugar solution (sp gr 1.270) was prepared according to the formula of Sheather (1923 a and b) and Benbrook (1925). Sugar solution having a specific gravity of 1.200 was made by adding 375 grams of sugar in 500 cc of water and 3.5 cc of phenol. A hydrometer was used to measure the specific gravity of all solutions at room temperature.

#### EXPERIMENTAL RESULTS

An examination of Table 1 shows that, in all tests in which a particular sample was inoculated with a known number of oöcysts and eggs, zinc sulfate consistently removed a larger number of oöcysts than the sugar solution (sp gr 1.270), while the latter, except in the case of soil sample No. 1, floated a few more helminth eggs. For every 100 oöcysts floated by the sugar solution (sp gr 1.270), 260 oöcysts were removed by the zinc sulfate solution, and for every 100 eggs floated by the zinc sulfate solution, 135 eggs were removed by the sugar solution. As regards sugar solution (sp gr 1.200), this medium proved to be slightly more efficient for the removal of eggs and slightly less efficient in recovering oöcysts than the zinc sulfate solution. For every 100 oöcysts floated by the sugar solution (sp gr 1.200), 114 oöcysts were floated by the zinc sulfate solution and for every 102 eggs floated by the zinc sulfate solution, 107 eggs were floated by the sugar solution (sp gr 1.200).

The foregoing results clearly demonstrate that each solution was more or less consistent in the relative number of eggs and oöcysts recovered from each of the samples, but was surprisingly inconsistent in the com-

TABLE 1.—*Flotation of eggs and oöcysts from soil (samples 1-5) and feces (samples 6-10)*

Sample No.	Av. inoculum per 2 cc sample	Zinc sulfate (sp. gr. 1.200)						Sugar (sp. gr. 1.270)						Sugar (sp. gr. 1.200)					
		Eggs			Oöcysts			Eggs			Oöcysts			Eggs			Oöcysts		
		No. <sup>1</sup>	Mean <sup>2</sup>	% Rec. <sup>3</sup>	No.	Mean	% Rec.	No.	Mean	% Rec.	No.	Mean	% Rec.	No.	Mean	% Rec.	No.	Mean	% Rec.
1	513	297 407 461	388	75.7	4,285 4,770 4,812	4,622	88.3	229 248 462 483	380	74.2	1,215 1,121 1,000 799	1,033	79.7	84 67 129 108 118 109	102 (σ <sub>m</sub> 9.4)	24.9	90 108 142 96 84 111	105 (σ <sub>m</sub> 8.4)	11.6
2	411	83 100 91 123 113 143	108 (σ <sub>m</sub> 9.1) <sup>4</sup>	26.5	108 122 126 115 132 147	130 (σ <sub>m</sub> 6.0)	11.3	289 201 210	233	42.6	139 144 172	151	8.5						
3	548	214 167 170	183	33.5	446 335 488	429	23.9	220 202 261	227		326 208 396	310							
4		100 93 110	101		387 302 490	393		235 191 237 141 255 269	221 (σ <sub>m</sub> 19.3)		177 336 370 129 491 320	300 (σ <sub>m</sub> 55.0)		223 206 223 273 259 239	237 (σ <sub>m</sub> 10.2)		674 467 499 479 753 759	605 (σ <sub>m</sub> 56.7)	
5		208 205 205 288 222 258	214 (σ <sub>m</sub> 9.8)		645 732 623 560 477 647	624 (σ <sub>m</sub> 42.8)													
6	1,875	243 139 281 281 294 256	249 (σ <sub>m</sub> 24.0)	13.2	1,036 701 794 1,125 974 963	927 (σ <sub>m</sub> 65.1)	10.8	107 83 149	113	25.8	61 102 81	81	5.7	387 263 344 299 287 241	303 (σ <sub>m</sub> 23.2)	16.2	762 710 751 828 923 820	801 (σ <sub>m</sub> 31.9)	9.3
7	438	72 49 95	72	16.4	110 172 140	140	9.9	1,289 1,155	1,222		141 141	141							
8		903 1,098	1,005		408 427	417													
9		0			4,144									1					4,036
10		885			1,355									657					1,367

<sup>1</sup> Each number represents the average number of oöcysts or eggs on first two coverslips.

<sup>2</sup> Mean counts for each sample.

<sup>3</sup> Percentage recovery.<sup>4</sup> Actual differences in the means divided by the differences in the standard deviations of the means.

parative percentages of oöcysts and eggs recovered from the different types of samples. Zinc sulfate removed approximately 75 per cent of the eggs and 88 per cent of the oöcysts from soil sample No. 1 and only 26 per cent of the eggs and 14 per cent of the oöcysts from soil sample No. 2 which was of the same origin as sample No. 1; the only difference in the two samples was that sample No. 2 had been dried for a longer period and had been inoculated with a different suspension of eggs and oöcysts. In contrast, zinc sulfate recovered 33 per cent of the eggs and 24 per cent of the oöcysts from soil sample No. 3 which had been collected from a poultry yard and contained approximately 3 times as much organic matter as soil samples 1 and 2. Similar inconsistencies were found in the percentage recoveries of eggs and oöcysts from the different samples with sugar solutions. However, the oöcyst returns of the sugar solution (sp gr 1.200) more closely approximated those of the zinc sulfate. The percentage recoveries of eggs and oöcysts from fecal samples were consistently lower than those from soil samples. The highest percentage recovery of eggs from any fecal sample was 25.8 when sugar solution having a specific gravity of 1.270 was used and the highest percentage of oöcysts was 10.8 when zinc sulfate was used.

Only the results obtained from samples inoculated with known numbers of oöcysts and eggs were used in drawing conclusions as to the comparative efficiency of recovery of eggs and oöcysts by the techniques used. The tests with the samples containing unknown numbers of eggs and oöcysts demonstrated the practicability of the techniques in that they showed the same proportional yield as with the known samples.

#### DISCUSSION

In an attempt to explain the variation in percentages of recovery of eggs and oöcysts from different samples by the same technique, such factors as organic matter content, the size of the soil particles, and moisture content, have been considered. As stated earlier in the paper, an analysis showed that the samples differed in the amount of organic matter present. The percentage recoveries of eggs and oöcysts in soil samples 1 and 2 were markedly different, yet they were taken from the same source and possessed the same organic matter content. Moreover, the size of the particles in the samples apparently did not account for the difference in the percentage recoveries of eggs and oöcysts, since no better returns were obtained from the finely ground samples than from the coarsely ground material. Furthermore, the amount of moisture present in the mixed samples, which would influence the concentration of the subsamples, apparently had little to do with the percentage recovery of eggs or oöcysts from the subsamples. Soil samples 1 and 2 contained almost the same amount of moisture (about 17 per cent) but upon flotation these

samples yielded very different returns of eggs and oöcysts. A higher percentage of eggs and oöcysts was recovered from soil sample 3 than from 2, yet sample 2 contained less moisture than sample 3. In regard to the suspensions used for inoculation, the separate cultures of eggs and oöcysts no doubt varied in degree of sporulation or embryonation. However, the condition of the cultures does not seem to explain the fact that the yields of eggs and oöcysts increased or decreased proportionately for the different samples.

In this study it was also found that the number of oöcysts and eggs present on the first two coverslips represented a large majority of the recoverable forms. More than 70 per cent of the oöcysts recovered by the first two coverslips were present on the first coverslip when zinc sulfate solution was used while only slightly more than 50 per cent of oöcysts recovered by the first two coverslips were present on the first coverslip when sugar solution of either specific gravity was used. The reverse of this seemed to be true for the eggs. About 78 per cent of the eggs were present on the first coverslip in the case of zinc sulfate solution while over 90 per cent of the eggs were present on the first coverslip in the case of sugar solution (sp gr 1.200). There was recovered on the first coverslip approximately 82 per cent of the eggs when sugar solution (sp gr 1.270) was used.

Granting that zinc sulfate was somewhat more efficient for the recovery of oöcysts in the present test and that sugar solution of the same specific gravity (1.200) was slightly more efficient for the recovery of eggs from fecal and soil samples, the sugar solution is preferred to the zinc sulfate solution for simultaneous flotation of these organisms because: (1) it is more easily obtained; (2) it does not crystallize so rapidly, thus obviating the necessity of examining the slides at once or placing them in a moist chamber; and (3) the eggs and oöcysts are more evenly distributed over the slide.

#### SUMMARY

A study of results obtained by a modified D.C.F. (direct centrifugal flotation) examination of soil and fecal samples containing known and unknown numbers of eggs and oöcysts of chicken parasites warrants the following conclusions:

1. Zinc sulfate solution of a specific gravity of 1.200 is a more efficient medium for the centrifugal flotation of oöcysts of the chicken coccidia, *Eimeria acervulina*, *E. mitis*, *E. maxima*, and *E. tenella*, than a sugar solution of a specific gravity of 1.270. However, the zinc sulfate is less efficient for floating the eggs of *Ascaridia lineata* and *Heterakis gallinae*.

2. Sugar solution of a specific gravity of about 1.200 is probably a more efficient medium for floating eggs, but zinc sulfate of the same



specific gravity is probably more efficient for floating oöcysts in simultaneous centrifugal flotations of the eggs and oöcysts of the chicken parasites mentioned.

3. Although sugar solution (sp gr 1.200), is no more efficient than zinc sulfate solution (sp gr 1.200), it is more practical for the detection of the above poultry parasites because (1) it is more easily obtained than zinc sulfate, (2) it is less expensive; and (3) it is a better mounting medium.

4. The modified D.C.F. technique used herein, recovered from 10 to 88 per cent of the eggs and oöcysts inoculated into soil and fecal samples. There is need for further study of the factors which determine the efficiency of simultaneous flotation of helminth eggs and protozoan cysts in order to improve the technique of examining fecal samples from poultry and other domesticated animals.

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## ORNITHODOROS PARKERI COOLEY: OBSERVATIONS ON THE BIOLOGY OF THIS TICK\*

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*Ornithodoros parkeri* was first collected in Natrona County, Wyoming, by the writer in 1934 and was described by Cooley in 1936. Subsequent observations on its biology and distribution and its relationship to the transmission of relapsing fever spirochetes and certain other infectious agents are summarized below. The host and distribution data have been mostly obtained from materials collected by staff members of the Rocky Mountain Laboratory, but a few of the records are from collections by field crews of the Plague Laboratory at San Francisco, California.

### DISTRIBUTION

Wyoming: Natrona, Carbon and Sweetwater Counties. Montana: Beaverhead, Madison and Ravalli Counties. Colorado: Moffat and Mesa Counties. Washington: Franklin, Yakima, Douglas and Okanogan Counties. Nevada: White Pine, Elko, Lyon, Lincoln, Clark and Churchill Counties. Utah: Uintah, Emery, Wayne, Washington, Grand, Carbon, and Iron Counties. California: Merced, Fresno and Kern Counties. Oregon: Benton and Umatilla Counties.

### TYPE AREAS

Sagebrush prairies, grassy slopes, and semi-desert areas, from low elevations, as the San Joaquin Valley in California, to elevations of 7000 feet on plateaus in southern Wyoming.

### HABITAT

Burrows and nests of rodents and burrowing owls.

### NATURAL HOSTS

Prairie dogs (*Cynomys* sp.), jack rabbits (*Lepus* sp.), cottontail rabbits (*Sylvilagus* sp.), mice (*Peromyscus* sp.), ground squirrels (*Citellus* spp.), weasel (*Mustela* sp.), and the burrowing owl (*Speotyto cunicularia*). This tick has been most frequently found in association with prairie dogs.

### EXPERIMENTAL HOSTS

These ticks feed readily on man, white mice, white rats, guinea pigs and monkeys.

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\* Contribution from the Rocky Mountain Laboratory (Hamilton, Montana) of the Division of Infectious Diseases of the National Institute of Health.

Four ticks, 3 males and one second stage nymph, have engorged on man experimentally; and one first stage nymph accidentally. There is no sensation while feeding. At the site of the bite there is a circumscribed hemorrhagic area with subsequent papule formation which may become necrotic. Intense itching occurs at intervals for 4 or 5 days.

#### FEEDING HABITS

Like *turicata* and *hermsi*, this species feeds rapidly. The male, when allowed to feed to repletion, becomes as fully distended as the female or any of the immature stages. The time required for engorgement depends on several factors, among which are: The interval which has elapsed since the last molt, the site of attachment, and the proximity of other attached ticks. Larvae, nymphs in all stages, and males and females may engorge in from 15 to 30 minutes. Feeding time determined for 20 males varied from 12 to 16 minutes.

#### COXAL FLUID

When engorgement is nearing completion, there is a copious amount of coxal fluid. It has not been shown that this fluid plays any essential rôle in the infective process in relapsing fever or spotted fever. Fluid from ticks shown to be infective for these diseases when injected into white rats or guinea pigs has not resulted in infection. In relapsing fever the host may become infected in less than one minute following attachment of the tick at a time when coxal fluid has not appeared.

#### LONGEVITY

The longevity of species of this genus is proverbial. Francis has recently reported the survival of *O. turicata* for 7 years. Late nymphs, males, and females of *parkeri* are still alive after approximately 4 years of fasting.

#### COPULATION TIME

The time was determined from the immobilization of the attached male to its voluntary detachment following the deposition of the spermatophore. Twenty-five such periods varied from 12 to 49 minutes. The average was 28 minutes.

#### MOLTING HABITS

Observations have been made on groups of *parkeri* from Montana, Wyoming, California, and Utah. When the larvae were engorged they were placed in individual cotton-stoppered shell glass vials, numbered serially, and kept at room temperature in a humidity jar containing a saturated solution of ammonium chloride. Higher temperatures hastened molting while low temperatures delayed it. Nymphs stored at a tempera-

ture of about 65° F failed to molt over a period of 3 months, but molted overnight when removed to a temperature of 97° F. Nymphs that are not completely engorged may molt regularly or may fail to molt until engorgement has taken place.

*Observation on Montana stock, Table 1.* September 11–13, 1937, 41 larvae engorged on a white rat. The limits for molting were 16 and 28 days, with the largest number molting by the 18th. Only 33 survived for the first nymphal feeding. The limits of nymphal molting were 14 and 41 days rather evenly distributed. In the second nymphal stage molting began on the 14th day and ended on the 31st, in the third it ranged from 15 to 32 days and in the fourth from 20 to 49 days. Twenty ticks survived to the adult stage. There were 16 males and 4 females. One of the males required only 3 nymphal stages to arrive to maturity while 15 males and the 4 females required 4 nymphal stages.

*Observations on Wyoming stock, Table 2.* July 28, 1938, 48 larvae engorged on a white rat. The molting period ranged from 8 to 12 days with 73 per cent molting on the eighth day. Only 35 ticks survived for the first nymphal feeding. The molting period varied from 13 to 18 days with the largest number molting on the 15th day. Thirty-four ticks survived for the second nymphal feeding. All but 2 molted in 14 to 19 days. These 2 molted on the 74th day. The third nymphal molts varied from 16 to 49 days, the fourth from 16 to 28 days, and one tick required a fifth molt which took place on the 25th day. Of the 34 ticks which survived to the adult stage 15 were males and 19 females. Three of the males and 2 females required only 3 nymphal stages, 12 males and 16 females, 4 nymphal stages, and one female 5 nymphal stages.

*Observations on California stock, Table 3.* December 4 to 6, 1939, 59 larvae engorged on white rats. All larvae molted in from 10 to 13 days with the largest number molting on the 11th day. Fifty-four ticks survived for the first nymphal feeding. The limits of the molting period were 11 and 19 days, with the largest number molting on the 14th. In the second nymphal stage the limits were 12 and 23 days, and in the third nymphal stage 13 and 20 days. Fifty-four ticks survived to the adult stage. Of these, 30 were males and 24 females. Eighteen of the males required only 2 nymphal stages, while 12 males and the 24 females required 3 nymphal stages. It is possible that the fewer nymphal stages required for maturity reflects the environmental conditions in central California.

*Observations on Utah stock, Table 4.* January 30 to February 20, 1940, 114 larvae engorged on white rats. The molting period varied from 7 to 13 days. Ninety-five ticks survived for the first nymphal feeding. The molting period ranged from 7 to 12 days. Ninety-three ticks survived for the second nymphal feeding. The molting period varied





TABLE 3.—*Ornithodoros parkeri* (California stock). Molting data. Larvae engorged December 4 to 6, 1939

Stage	Numerators: number of days after feeding required for molting. Denominators: number of ticks that molted in that number of days.										Total ticks
Larval .....	$\frac{10}{20}$	$\frac{11}{27}$	$\frac{12}{8}$	$\frac{13}{4}$							59
First nymphal .	$\frac{11}{1}$	$\frac{12}{2}$	$\frac{13}{10}$	$\frac{14}{21}$	$\frac{15}{7}$	$\frac{16}{7}$	$\frac{17}{2}$	$\frac{18}{3}$	$\frac{19}{1}$		54
Second nymphal	$\frac{12}{1}$	$\frac{13}{2}$	$\frac{14}{6}$	$\frac{15}{7(2\delta\delta)}$	$\frac{16}{10(2\delta\delta)}$	$\frac{17}{5(1\delta)}$	$\frac{18}{11(3\delta\delta)}$	$\frac{19}{2\delta\delta}$	$\frac{20}{5(4\delta\delta)}$	$\frac{21}{2\delta\delta}$	53 (18 $\delta\delta$ )
Third nymphal .	$\frac{13}{1\delta}$	$\frac{14}{3\delta\delta}$	$\frac{15}{5\delta\delta}$	$\frac{16}{4\delta\delta}$	$\frac{17}{8\delta\delta}$	$\frac{18}{5\delta\delta}$	$\frac{19}{4\delta\delta}$	$\frac{20}{3\delta\delta}$	$\frac{21}{1\delta}$		36 (12 $\delta\delta$ , 24 $\delta\delta$ )

TABLE 4.—*Ornithodoros parkeri* (Utah stock). Molting data. Larvae engorged January 30 to February 20, 1940

Stage	Numerators: number of days after feeding required for molting. Denominators: number of ticks that molted in that number of days.										Total ticks
Larval .....	$\frac{7}{6}$	$\frac{8}{32}$	$\frac{9}{50}$	$\frac{10}{19}$	$\frac{11}{5}$	$\frac{12}{1}$	$\frac{13}{1}$				114
First nymphal ....	$\frac{7}{10}$	$\frac{8}{56}$	$\frac{9}{23}$	$\frac{10}{3}$	$\frac{11}{2}$	$\frac{12}{1}$					95
Second nymphal ....	$\frac{10}{10}$	$\frac{11}{25}$	$\frac{12}{32}$	$\frac{13}{4}$	$\frac{14}{8}$	$\frac{15}{4}$	$\frac{16}{1}$	$\frac{17}{24}$	$\frac{18}{1}$	$\frac{19}{34}$	93
Third nymphal ....	$\frac{15}{1}$	$\frac{16}{3}$	$\frac{17}{12}$	$\frac{18}{6(1\delta)}$	$\frac{19}{13(3\delta\delta)}$	$\frac{20}{13(6\delta\delta)}$	$\frac{21}{15(7\delta\delta)}$	$\frac{22}{8(5\delta\delta)}$			93 (30 $\delta\delta$ , 11 $\delta\delta$ )
Fourth nymphal ....	$\frac{24}{2\delta\delta}$	$\frac{25}{1\delta}$	$\frac{26}{1\delta}$	$\frac{27}{31}$	$\frac{28}{1\delta}$	$\frac{29}{2\delta\delta}$	$\frac{30}{1\delta}$	$\frac{31}{45}$	$\frac{32}{48}$	$\frac{33}{57}$	52 (19 $\delta\delta$ , 32 $\delta\delta$ ) (1 died with- out molting)

from 10 to 34 days. In the third nymphal stage, molting began on the 15th day and was complete on the 41st. Forty-one ticks, 30 males and 11 females, reached the adult stage at this time. Fifty-two ticks remained for the fourth nymphal feeding. Molting began on the 24th day and was complete on the 68th. One tick died without molting. Of the 51 remaining ticks, 19 were males and 32 females—a total of 49 males and 43 females.

*Summary of molting observations, Table 5.* The duration of the larval stage for 262 ticks was 7 to 28 days; of the first nymphal stage for 217 ticks, 7 to 41 days; of the second nymphal stage for 209 ticks, 10 to 74 days; of the third nymphal stage for 192 ticks, 13 to 49 days; of the fourth nymphal stage for 98 ticks, 16 to 59 days; and for one tick in the fifth nymphal stage, 25 days. Of 198 ticks reared to adults, 18 males required 2 nymphal stages; 82 (45 ♂♂, 37 ♀♀), 3 nymphal stages, 97 (46 ♂♂, 51 ♀♀), 4 nymphal stages, and one female, 5 nymphal stages.

#### RELATION TO INFECTIOUS DISEASES

*Relapsing fever.* This species has not been definitely connected with human infection. However, it feeds readily on man and is the only species known in areas in which at least 17 cases have originated. Twelve specimens were collected from an endemic area in western Nevada. Spirochetes have been recovered from ticks collected in two areas in Wyoming, and one each in Montana, Utah, California, and Nevada. The Wyoming, Montana, and Utah strains have been studied in white mice, white rats, guinea pigs, and rhesus monkeys, the California strain only in white mice. Rhesus monkeys have not been shown to be susceptible. The other 3 hosts show typical relapses in varying degrees. In guinea pigs the reappearance of spirochetes in the peripheral blood is accompanied by clinical relapses.

*Rocky Mountain spotted fever.* *O. parkeri* has not been found infected in nature but ticks which have fed on infected guinea pigs readily produce the disease when later allowed to feed on fresh guinea pigs. Nymphs, males and females transmit the infecting agent. Two females have been shown to be infective 685 and 739 days, respectively, following the infective feeding in the second nymphal stage, and generation-to-generation transmission has been demonstrated.

*American Q fever (Nine Mile fever).* The causative organism (*Rickettsia diaporica*) survives and retains its virulence for many months in the tissues of successive stages of *O. parkeri*, but is not transmitted during the process of feeding. The tick excrement is infective.

*Tularaemia.* *B. tularensis* survives and retains its virulence for at least 701 days, but is not transmitted during the process of feeding.

TABLE 5.—*Ornithodoros parkeri*. Summary of molting observations. Figures outside parenthesis, 16-28 = limits of respective stages in days; figures in parenthesis, (41) = number of ticks in respective stage

Stock	Larvae	1st nymphs	2d nymphs	3d nymphs	4th nymphs	5th nymphs	Adults
Montana .....	16-28(41)	14-41(33)	14-31(28)	15-32(19)	20-49(18)		19 16♂♂, 3♀♀
Wyoming .....	8-12(48)	13-18(35)	14-74(34)	16-49(34)	16-28(29)	25(1)	33 14♂♂, 19♀♀
California .....	10-13(59)	11-19(54)	12-23(54)	13-20(36)			54 30♂♂, 24♀♀
Utah .....	7-13(114)	7-12(95)	10-34(93)	15-21(93)	24-59(51)		92 49♂♂, 43♀♀
Summary .....	7-28(262)	7-41(217)	10-74(209)	13-49(182)	16-59(98)	25(1)	
Adults following re- spective molt ..			18♂♂	82(45♂♂, 37♀♀)	97(46♂♂, 51♀♀)	1♀	198 109♂♂, 89♀♀

## CANNIBALISM

The phenomenon of one tick feeding on another of the same species has been termed cannibalism, which is obviously a misnomer as the tick host is neither *consumed* nor sufficiently harmed to prevent normal functions. The two following observations were made: August 30, 1939, a last stage nymph feeding on a guinea pig was punctured through the dorsum by a second tick which proceeded to completely engorge. The "host" tick molted normally to a female, copulated and oviposited; there were 198 eggs, 46 larvae. August 31, 1939, another last stage nymph was similarly punctured and the "host" tick again completely engorged. The latter molted normally to a female, copulated and oviposited. Eggs, 190; larvae, 82.

Similar observations on *O. turicata* were made by Wood (1912) and Francis (1938) and on *O. talaje* (unpublished notes, Davis, 1939b).

TABLE 6.—*Ornithodoros parkeri*: oviposition data

Female No.	Stock	Date of engorgement	Date of copulation	First eggs	First larvae	Total eggs	Total larvae
25	Wyoming	5-22-39	5-22-39	8-12-39	8-27-39	156	145
42	"	5-19-39	5-19-39	6-12-39	6-30-39	191	37
18	"	5-11-39	5-11-39	6- 4-39	6-21-39	186	10
48	"	11-14-39	11-14-39	1-16-40	2- 8-39	331	147
4	"	9- 7-39	9- 7-39	9-29-39	11-18-39	158	81
2	"	10-12-39	10-12-39	12-18-39	1-10-39	134	15
2	"	1-19-40	Unknown	2- 5-40	2-21-40	181	116
4	Utah	9-20-39	9-20-39	11-22-39	12-13-39	377	293
4	"	12-22-39	12-22-39	1-11-40	1-25-40	336	162
2	Nevada	11-30-39	Unknown	2- 2-40	2-19-40	171	104
2	Washington	11-15-39	11-16-39	3- 1-40	3-17-40	190	82
9	"	11-13-39	11-13-39	3-12-40	3-30-40	198	46
4	California	10-18-39	10-18-39	11- 4-39	11-26-39	276	71

## OVIPOSITION (TABLE 6)

*O. parkeri* has not been observed to oviposit prior to copulation. Following engorgement and copulation, it oviposits less regularly than *turicata* under similar laboratory conditions. In Wyoming and Montana stock, which we have used most extensively, there is a marked tendency toward a rest period through the winter and early spring months. Table 6 shows the dates of engorgement and copulation, the appearance of the first eggs and of the first larvae, with the total number of eggs and larvae for each of 11 females. It includes data on Wyoming, Utah, Nevada, Washington, and California stock. In the case of the female from Utah, and one from Wyoming, data for 2 successive ovipositions are given. The shortest interval between engorgement and oviposition was 17 days and the longest 120. The largest number of eggs deposited at one oviposition was 377 and the average 222.

## SUMMARY

Data on the distribution, type areas, habitats, and natural and experi-

mental hosts of the argasid tick, *Ornithodoros parkeri*, are given and observations on feeding habits, coxal fluid, longevity, copulation, molting, oviposition, cannibalism and its relation to relapsing fever, Rocky Mountain spotted fever, American Q fever and tularaemia are presented.

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A NEW SPECIES OF *ORNITHODOROS* TICK FROM  
NEVADA (ACARINA: IXODOIDEA)

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*Ornithodoros cooleyi* n. sp.

(Figs. 1-5)

ADULT FEMALE. (Male not known.)

*Body*: Length 12 mm, width 7 mm. Ovate-oblong, somewhat narrowed and with margin rather sharply rounded anteriorly, hood not visible dorsally. Integument medium thick, sandy in color in the unengorged specimen. True discs absent, unmammillated tracts present, appearing as furrows in the unengorged tick. Numerous pit-like depressions present. Microscopically, integument irregularly mammillated, mammillae surrounded by sinuous grooves. Dorsally, sculpturing consists of rather shallow, bilaterally arranged depressions with a shallow median posterior groove, anterior marginal groove indistinct and broadly U-shaped. Margin of body rounded in unengorged specimen. Ventrally, integument similar to that of dorsum. Eyes lacking. Sub-coxal and supra-coxal folds not well marked. Supra-coxal folds extending anteriorly to form the margins of the hood, supra-coxal grooves extending laterally to coxae IV, expanding into triangular depressions surrounding the spiracles. Supra-coxal folds not diverging to form a notch in the lateral margins of the body. Sub-coxal folds originating at the base of coxae II expanding and extending to coxae IV. Median post-anal groove not well marked. Transverse pre-anal and post-anal grooves absent.

*Anus*: Ovate.

*Spiracles*: Located in triangular expansions of the supra-coxal grooves dorsal and slightly anterior to coxae IV. Spiracle circular, with a circular plate.

*Hood*: Large, broadly pointed anteriorly.

*Capitulum*: Placed in a poorly defined camerastome, with few hairs.

*Hypostome*: Slightly lanceolate, of fair size, apex emarginate. Dentition: Crowned with three or four semi-circular rows of minute teeth followed by two semi-circular rows of slightly larger teeth. Principal dentition consisting of two bilateral rows of five large teeth. Posterior to these a group of five smaller teeth.

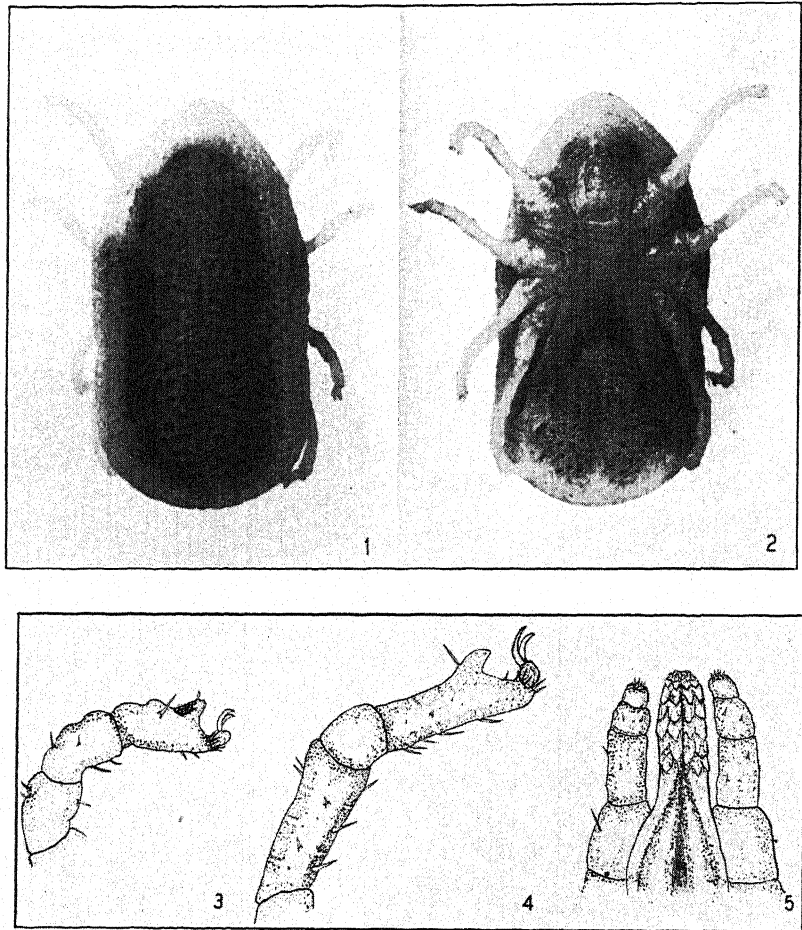
*Palpi*: Comparatively long and slender.

*Legs*: Slender, coxae progressively smaller from I to IV. Coxae II, III and IV contiguous; coxae I and II separated. Tarsus I equipped with three well defined diagnostic protuberances with a Haller's organ separating the distal two; claws surrounded by spines. Tarsus IV equipped with one well defined protuberance above the claws, claws surrounded by spines, one lanceolate spine present at the apex of the distal protuberance, numerous hairs present on the tarsus.

*Locality*: Taken near Rox, Lincoln County, Nevada.

*Holotype*: Deposited in the California Academy of Sciences, Golden Gate Park, San Francisco.

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*Ornithodoros cooleyi* n. sp. adult female

FIG. 1. Dorsal view. FIG. 2. Ventral view. FIG. 3. Tarsus of 1st leg.  
FIG. 4. Tarsus of 4th leg. FIG. 5. Hypostome and palpi.

THE RELATION OF PHYSID AND PLANORBID SNAILS TO  
THE LIFE CYCLE OF THE STRIGEID TREMATODE,  
*COTYLURUS FLABELLIFORMIS* (FAUST, 1917)<sup>1</sup>

W. W. CORT, LOUIS OLIVIER, AND STERLING BRACKETT

The first account of any stage in the life cycle of the duck strigeid, *Cotylurus flabelliformis*, was given by Faust (1917a and b), who described as *Cercaria flabelliformis* a strigeid metacercaria which he found parasitic in rediae in *Physa gyrina* Say. These rediae also contained daughter rediae, and at the time Faust thought that they belonged to the life cycle of the strigeid. Hughes (1929) gave a more complete description of Faust's metacercaria and called it *Tetracotyle flabelliformis*. His material came from 9 specimens of *Lymnaea stagnalis perampla* Walker and from one specimen of *Helisoma campanulatum smithii* (Baker). He noted that in the planorbid some of the tetracotyles were inside the sporocysts of another trematode. Cort (1917) gave a preliminary description of a strigeid from a species of *Physa* and named it *Cercaria douglasi*, and Cort and Brooks (1928) identified as *C. douglasi* cercariae found in *Stagnicola emarginata angulata* (Sowerby), *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla*, and *Physa parkeri* Currier. Their description was actually made from the cercariae obtained from three specimens of the varieties of *L. stagnalis*. Van Haitsma (1931) fed tetracotyles which he identified as *T. flabelliformis*, to domestic ducks and obtained adult strigeids belonging to the genus *Cotylurus*. The name of the species, therefore, became *C. flabelliformis* (Faust, 1917). Most of the tetracotyles used by Van Haitsma in his feeding experiments came from natural infections of lymnaeid snails although he also used a natural infection from *Helisoma trivolvis* (Say). In one of his experimental infections he utilized metacercariae which had developed in the varieties of *L. stagnalis* from experimental infections with the cercariae from these same hosts. It has recently been shown (Olivier and Cort, 1941) that the cercariae described by Cort (1917) and Cort and Brooks (1928) are actually two distinct species: *C. douglasi* Cort, 1917 (nec Cort and Brooks, 1928) which occurs only in *Physa* spp. and the cercaria of *C. flabelliformis* (= *C. douglasi* Cort and Brooks, 1928) which parasitizes only lymnaeids.

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<sup>1</sup> From the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is the third of a series on the researches on the second intermediate host relations of *Cotylurus flabelliformis* carried out in the Douglas Lake region beginning with the summer of 1932. The first paper in this series was by Winfield (1932) and the second by Nolf and Cort (1933).

It can be seen from the discussion given above, that the metacercarial stage of *C. flabelliformis* has been reported from snails belonging to three families, viz., LYMNAEIDAE, PHYSIDAE, and PLANORBIDAE. We found, however, in a series of preliminary experiments that in many cases development of the metacercariae could not be obtained in physid and planorbid snails exposed to the cercariae of *C. flabelliformis*, whereas lymnaeids were always successfully infected. It was decided, therefore, to carry out further experiments on the relations of these snails to the life cycle of this strigeid. In all, 13 experiments were performed in the summers of 1935, 1936, 1937, and 1940. The results, without exception, showed that physid and planorbid snails, negative for cercarial infections, were entirely unsuitable second intermediate hosts for the development of the metacercariae of *C. flabelliformis*. When, however, these snails were infected with the sporocysts and rediae of certain species of cercariae, the metacercariae of *C. flabelliformis* developed normally in them to the tetracotyle stage.

#### MATERIALS AND METHODS

Water containing cercariae of *C. flabelliformis* that had escaped from infected specimens of the varieties of *L. stagnalis* was poured into aquaria containing lymnaeid snails, for controls, and either physids or planorbids some of which were known to harbor infections of cercariae. Large numbers of the cercariae of *C. flabelliformis*, usually from several snails, were used, and frequently the exposure was continued for several days. In every experiment some of the snails died before the date of examination. These are entirely omitted from the protocols. Before the controls were placed in the experiments, examinations of some of the snails from the same collections were made for natural infections of tetracotyles; and before the experimental physids or planorbids were put in some of them were also examined.

The experiments were terminated from about 10 days to a month after the last exposure to the cercariae. At the time of examination each snail was carefully removed from its shell and all parts except the foot were teased apart as completely as possible. Since the metacercariae of *C. flabelliformis* normally develop in the hermaphroditic gland, the whole digestive gland in which this organ is embedded was always most carefully examined. The metacercariae were then counted and the stage of development recorded. The counts in the protocols are obviously always somewhat less than the actual numbers of the metacercariae present, since it is almost impossible to tease apart all the snail tissues to such a degree that all of them would be freed.

The developmental stages of the metacercariae of *C. flabelliformis* have never been described but the morphology of the same stages has been given for the closely related European species, *Cotylurus cornutus*



(Rud.) (cf. Szidat, 1924, and Wesenberg-Lund, 1934, Pl. XXV). When the cercaria penetrates into the snail it loses its tail and the body soon begins to enlarge. In about 10 days it has grown five to six times the length of the cercarial body and has become very broad and thick. The internal structures become very loosely organized and the space is occupied by stellate cells widely separated by large fluid-filled spaces. After maximum size is reached the tissues begin to be reorganized and condensed, the size decreases, and the hold-fast organ, the lateral cups, and other structures of the tetracotyle are formed. During this reorganization the cystogenous glands become prominent and a narrower hind-body is set off from the fore-body. The fully formed metacercaria (tetracotyle) is about one-third the length of the largest stage and is surrounded by a secreted cyst. The metacercariae recovered in our experiments were classified into three groups according to age, viz., (1) "developing" (all stages up to the largest size and those beginning to reorganize), (2) "pre-cysts" (those with a distinct hind-body but without any cyst wall), (3) "cysts" (those surrounded by a cyst wall).

Protocols for only eight of the thirteen experiments are included since five were unsatisfactory due to death of controls, lack of previous examination of controls or experimental snails for natural infection, or because of the death of too large a proportion of the experimental snails that harbored cercarial infections.

The following planorbid and physid snails were used in the experiments:

*Helisoma trivolvis* (Say)  
*H. campanulatum smithii* (Baker)  
*H. antrosum percarinatum* (Walker)  
*Planorbula armigera* (Say)  
*Physa gyrina* Say  
*P. parkeri* Currier

The lymnaeid controls were:

*Lymnaea stagnalis appressa* Say  
*L. stagnalis parampla* Walker  
*Stagnicola palustris elodes* (Say)

The following larval trematode infections were found in the examinations of the experimental snails:

Cer. *Clinostomum marginatum* (Rud.) (cf. Hunter and Hunter, 1935; Krull, 1934a).

Cer. *Macroderoides typicus* (Winfield) (cf. McMullen, 1935).

Cer. *Petasiger nitidus* Linton (cf. Beaver, 1939).

Cer. *Triganodistomum mutabile* (Cort) (= *Cercariaeum mutabile* Cort, 1918) (cf. Wallace, 1939).

*Cer. Apatemon sphaerocephalus* (= *Cercaria burti* Miller, 1923) (cf. Willey and Rabinowitz, 1938).

*Cercaria dohema* Cort and Brackett, 1937.

*Cer. Psilostomum ondatrae* Price (cf. Beaver, 1939).

*Cer. Alaria intermedia* (Olivier and Odlaug, 1938) (cf. Odlaug, 1940).

*Cercaria physae* Cort and Brooks, 1928.

*Cer. Uvulifer ambloplitis* (Hughes) (= *Cercaria bessiae* Cort and Brooks, 1928) (cf. Krull, 1934 b; Hunter and Hunter, 1935).

*Cercaria elephantis* Cort, 1917.

PROTOCOLS OF EXPERIMENTS IN WHICH SNAILS OF THE FAMILIES PLANORBIDAE AND  
PHYSIDAE WERE EXPOSED TO THE CERCARIAE OF *Cotylurus flabelliformis*

EXPERIMENT 1

Experimental snails: *H. trivolvis*.

Examination for natural infections: (none made)

Exposed to cercariae of *C. flabelliformis*, 6/23-7/28/37.

23 surviving snails examined, 7/28/37.

Results of examination:

20 without cercarial infection:

14 had no metacercariae.

6 had a total of 15 metacercariae (4 cysts, 2 pre-cysts, 9 developing).

2 with *cer. C. marginatum*:

188 and 64 metacercariae (52 cysts, 200 pre-cysts and developing).

1 with *cer. M. typicus*:

162 metacercariae (62 cysts, 100 developing).

Control snails: *L. stagnalis appressa* and *L. stagnalis perampla*.

Examination for natural infection:

20 without cercarial infection:

17 had no metacercariae

3 had a total of 4 metacercariae (cysts)

Examination for experimental infection:

8 without cercarial infection:

68-779, ave. 519 metacercariae (18% cysts, 1% pre-cysts, 81% developing).

Comment: One of the controls had an infection of the cercaria of *C. flabelliformis* which came to maturity during the course of the experiment. This accounts for the heavy metacercarial infections and for the presence of all stages of development.

EXPERIMENT 2

Experiment snails: *H. campanulatum smithii*.

Examination for natural infection: (none made)

Exposed to cercariae of *C. flabelliformis*, 6/23-7/2/37.

12 surviving snails examined, 7/13/37.

Results of examination:

5 without cercarial infection:

2 had no metacercariae.

3 had a total of 6 metacercariae (2 cysts, 4 developing).

4 with *cer. M. typicus*:

7, 9, 27, and 37 metacercariae (8 cysts, 35 pre-cysts, 32 developing).

1 with *cer. C. marginatum*:

3 metacercariae (cysts).

1 with *cer. P. nitidus*:

17 metacercariae (3 pre-cysts, 14 developing).

- 1 with both cer. *M. typicus* and cer. *U. ambloplitis*:  
19 metacercariae (pre-cysts).
- Control snails: *L. stagnalis appressa* and *L. stagnalis perampla*.  
Examination for natural infection: (same as experiment 1).
- Examination for experimental infection:
  - 7 without cercarial infection:  
26-211, ave. 105 metacercariae (1% cysts, 28% pre-cysts, 71% developing).

## EXPERIMENT 3

- Experimental snails: *H. antrosom percarinatum*.  
Examination for natural infection:
  - 14 without cercarial infection:  
no metacercariae.
  - 5 with cer. *T. mutabile*:  
no metacercariae.
  - 1 with *C. dohema*:  
no metacercariae.
- Exposed to cercariae of *C. flabelliformis*, 8/1-3/40.  
41 surviving snails examined, 8/11-13/40.
- Results of examination:
  - 25 without cercarial infection:  
no metacercariae.
  - 10 with cer. *T. mutabile*:  
11-153, ave. 88 metacercariae (about 40 cysts, a number of pre-cysts, majority developing).
  - 5 with cer. *A. sphaerocephalus*:  
55-218, ave. 124 metacercariae (about same stages as in snails infected with cer. *T. mutabile*).
  - 1 with *C. dohema*:  
44 metacercariae (a few pre-cysts, most developing).
- Control snails: *S. palustris elodes*.  
Examination for natural infection:
  - 14 without cercarial infection:  
no metacercariae.
- Examination for experimental infection:
  - 9 without cercarial infection:  
20-268, ave. 156 metacercariae (all developing).
- Comment: The controls from all the 1940 series of experiments were specimens of *S. palustris elodes* from the same collection. Therefore, the examinations for natural infections of controls will be the same in all.

## EXPERIMENT 4

- Experimental snails: *H. antrosom percarinatum*.  
Examination for natural infection:
  - 5 without cercarial infection:  
no metacercariae.
  - 1 with cer. *P. ondatrae*:  
no metacercariae.
  - 1 with unidentified xiphidio-cercariae:  
no metacercariae.
- Exposed to cercariae of *C. flabelliformis*, 8/12-20/36.  
59 surviving snails examined, 8/28-29/36.
- Results of examination:
  - 47 without cercarial infection:  
no metacercariae.

4 with cer. *A. sphaerocephalus*:

538-751, ave. 631 metacercariae (3 pre-cysts, all the rest developing).

4 with cer. *P. ondatrae*:

46-410, ave. 216 metacercariae (all developing).

2 with cer. *T. mutabile*:

0 and 30 metacercariae (developing).

2 with cer. *M. typicus*:

5 and 142 metacercariae (developing).

Control snails: none used.

#### EXPERIMENT 5

Experimental snails: *P. armigera*.

Examination for natural infection:

16 without cercarial infection:

no metacercariae.

Exposed to cercariae of *C. flabelliformis*, 7/21-24/40.

76 surviving snails examined, 8/10/40.

Results of examination:

72 without cercarial infection:

no metacercariae.

2 with unidentified xiphidio-cercaria:

79 and 249 metacercariae (mostly cysts, a few pre-cysts and developing).

2 with cer. *A. intermedia*:

224 and 298 metacercariae (a few cysts and pre-cysts, most developing).

Control snails: *S. palustris elodes*.

Examination for natural infection:

(same as experiment 3).

Examination for experimental infection:

6 without cercarial infection:

126-383, ave. 236 metacercariae (1% cysts, 6% pre-cysts, 93% developing).

#### EXPERIMENT 6

Experimental snails: *P. gyrina*.

Examination for natural infection:

13 without cercarial infection:

10 had no metacercariae.

3 had a total of 16 metacercariae (10 pre-cysts, 6 developing).

9 with *C. physae*:

no metacercariae.

3 with echinostome cercaria:

2 had no metacercariae.

1 had 1 metacercaria (pre-cyst).

Exposed to cercariae of *C. flabelliformis*, 7/25-29/40.

82 surviving snails examined, 8/12-13/40.

Results of examination:

36 without cercarial infection:

21 had no metacercariae.

15 had a total of 44 metacercariae (37 cysts, 7 pre-cysts).

27 with *C. physae*:

5-452, ave. 159 metacercariae (62% cysts, 33% pre-cysts, 5% developing).

12 with echinostome cercaria:

9-459, ave. 86 metacercariae (almost all encysted).

4 with reniferid cercaria:

0, 122, 217, and 265 metacercariae (96% cysts, 4% pre-cysts).

3 with cer. *A. sphaerocephalus*:

20, 80, and 217 metacercariae (96% cysts, 4% pre-cysts).

Control snails: *S. palustris elodes*.

Examination for natural infection:

(same as experiment 3).

Examination for experimental infection:

2 without cercarial infection:

31 and 224 metacercariae (3% pre-cysts, 97% developing).

Comment: The natural infection of the experimental snails was probably the metacercarial stage of *C. douglasi* (see discussion). *C. douglasi* was found in the summer of 1940 in one specimen of *P. gyrina* from the beach where the experimental snails used in experiment 6 were collected.

The echinostome cercariae in this experiment and experiment 8 probably belonged either to *Echinostoma revolutum* or *Echinoparyphium recurvatum*, the cercariae of which are difficult to distinguish and which are both found in the species of *Physa* in the region.

The reniferid cercariae probably belonged to one of the two species for which the life cycles were described by Talbot (1933). As noted by this author it is almost impossible to make accurate identifications of these species from the cercaria only.

#### EXPERIMENT 7

Experimental snails: *P. gyrina*.

Examination for natural infection:

13 without cercarial infection:

no metacercariae.

Exposed to cercariae of *C. flabelliformis*, 7/30/40.

27 surviving snails examined, 8/14/40.

Results of examination:

25 without cercarial infection:

no metacercariae.

1 with *C. physae*:

346 metacercariae (all cysts).

1 with unidentified xiphidio-cercaria:

46 metacercariae (all cysts).

Control snails: *S. palustris elodes*.

Examination for natural infection:

(same as experiment 3).

Examination for experimental infection:

5 without cercarial infection:

13-185, ave. 96 metacercariae (3% cysts, 73% pre-cysts, 24% developing).

#### EXPERIMENT 8

Experimental snails: *P. parkeri* (large juveniles).

Examination for natural infection:

5 without cercarial infection:

4 had no metacercariae.

1 had 3 metacercariae (pre-cysts) (probably *C. douglasi*).

2 with *C. physae*:

no metacercariae.

3 with echinostome cercaria:

no metacercariae.

Exposed to cercariae of *C. flabelliformis*, 7/31-8/1/40.

57 surviving snails examined, 8/14/40.

Results of examination:

29 without cercarial infection:

26 had no metacercariae.

3 had 4 metacercariae (cysts) (probably *C. douglasi*).



- 27 with echinostome cercaria :
  - 1 had no metacercariae.
  - 26 had 1-152, ave. 71 metacercariae (99% cysts, 1% pre-cysts).
- 1 with *C. physae* :
  - 109 metacercariae (cysts).
- Control snails : *S. palustris elodes*.
  - Examination for natural infection :
    - (same as experiment 3).
  - Examination for experimental infection :
    - 5 without cercarial infection :
      - 7-92, ave. 38 metacercariae (37% cysts, 20% pre-cysts, 43% developing).

RESULTS OF EXAMINATION OF *H. trivolvis* NATURALLY EXPOSED TO  
INFECTION WITH THE CERCARIAE OF *C. flabelliformis*

A collection of *Helisoma trivolvis* was made during the summer of 1940 from an area on Douglas Lake where large numbers of the varieties of *L. stagnalis* lived in the same environment. Some of these lymnaeids were infected with the cercaria of *C. flabelliformis* since six specimens infected with this species appeared in about 300 snails examined. It seemed evident, therefore, that the snails in this area were being exposed to infection with this cercaria.

RESULTS OF EXAMINATION OF *H. trivolvis* (128 SPECIMENS OF LARGE AND MEDIUM  
SIZED ADULTS) FOR NATURAL INFECTIONS WITH METACERCARIAE  
OF *C. flabelliformis*

- 106 without cercarial infection :
  - no metacercariae.
- 12 with cer. *C. marginatum* :
  - 2 had no metacercariae.
  - 10 had 1-20, ave. 8 metacercariae.
- 4 with cer. *M. typicus* :
  - 0, 29, 87, and 131 metacercariae.
- 3 with cer. *A. sphaerocephalus* :
  - 26, 48, 434 metacercariae.
- 1 with cer. *U. ambloplitis* :
  - 38 metacercariae.
- 1 with *C. elephantis* :
  - 518 metacercariae.
- 1 with immature stylet :
  - no metacercariae.

Comment: Most of these metacercariae were fully developed tetracotyles, but there were also small numbers in all stages of development.

DISCUSSION

The data given above indicate that four species of planorbid and two of physid snails are unsuitable as second intermediate hosts for *C. flabelliformis* unless they harbor infections with certain species of cercariae. It is also evident from the experiments that the varieties of *L. stagnalis* and *S. palustris elodes* are normal second intermediate hosts for this species. In fact we have found that the metacercariae of this strigeid

will develop in all the species of lymnaeid snails found in the Douglas Lake region, whether or not they are infected with larval trematodes.<sup>2</sup> Snails of the family LYMNAEIDAE, therefore, can be considered as the normal second intermediate hosts of *C. flabelliformis*.

When the development does occur in the physid and planorbid snails it appears to be entirely normal. In fact, as mentioned earlier, Van Haitsma obtained adults of *C. flabelliformis* by feeding tetracotyles from a planorbid snail to a domestic duck. When physid and planorbid snails are exposed to the cercariae of *C. flabelliformis*, penetration occurs whether or not the snails are infected with species of cercariae. In those that are uninfected the cercarial bodies do not develop and soon die. On one occasion three juveniles of *P. parkeri*, one adult of *H. antrosom percarinatum*, and one juvenile of *Stagnicola emarginata angulata* were placed in a small dish with large numbers of the cercariae of *C. flabelliformis* and were observed under a binocular microscope. The cercariae were seen to penetrate readily into all five snails and in a few minutes numerous detached tails were found in the dish. Four days later when these snails were examined numerous dead cercarial bodies were found in the specimens of *Physa* and *Helisoma*. The specimen of *S. emarginata angulata* contained numerous living cercarial bodies. Also, in one of the experiments with *P. gyrina*, presented above, it was observed that the snails showed great irritation after unusually large numbers of cercariae of *C. flabelliformis* had been poured into the aquarium. They reacted by moving the shell rapidly back and forth. This same reaction frequently occurs when lymnaeid snails are exposed to large numbers of these cercariae, and seems to result from the irritation produced by their penetration.

In the physid and planorbid snails that had been successfully infected with larvae of *C. flabelliformis*, metacercariae were always found inside the sporocysts and rediae. When sporocysts were present a large proportion of the metacercariae were also found free in the dish probably because the sporocysts were always broken up by the teasing of the digestive gland. In the infections with rediae (*C. marginatum*, *T. mutabile*, *P. ondatrae*, and the echinostomes) only a small proportion of the metacercariae were ever found outside the rediae. This seemed to be due to the fact that few of the rediae were broken in the teasing of the preparations. Also of interest in this connection is the specimen of *P. gyrina* in experiment 7, infected with an unidentified xiphidiocercaria. In this snail all of the sporocysts were in a clump well in front of the digestive gland. Examination showed that all the metacercariae were in these sporocysts and not a single one was found elsewhere. From this evidence we conclude that in these abnormal hosts the metacercariae of *C. flabelliformis*

<sup>2</sup> Further discussion of these experiments will be reserved for a later paper.

develop inside the sporocysts and rediae and, therefore, exhibit a form of hyperparasitism.

The metacercariae of *C. flabelliformis* may also be hyperparasites in normal hosts that harbor infections with various species of cercariae. In fact in our experimental infections of lymnaeid snails with this cercaria we have found the metacercariae inside sporocysts and rediae whenever they were present. Also, in the literature on larval trematodes, strigeid metacercariae, especially the fully developed tetracotyles, have frequently been reported and figured inside sporocysts and rediae. Such observations are recorded in some of the earliest studies on trematode development.<sup>3</sup>

Another significant point is that the development of the metacercarial stage inside sporocysts and rediae in an abnormal host is completed more rapidly than in the hermaphroditic gland of a normal host without cercarial infection. While this is suggested in almost all of the experiments, it is shown most clearly in experiments 7 and 8, in which most of the metacercariae were encysted after two weeks in the physids harboring rediae or sporocysts while in the controls the majority were still in the "developing" or "pre-cyst" stages. In normal hosts also, the development of the metacercariae is more rapid inside the sporocysts and rediae of infected individuals than in the hermaphroditic gland of those harboring no cercarial infections.

A further point shown in the protocols is that a few metacercariae, including mature tetracotyles, were present in several physid and planorbid snails that harbored neither rediae nor sporocysts. Those found in the physids were probably the metacercariae of *C. douglasi*, which is present in species of *Physa* in this region and which uses these same snails as normal second intermediate hosts. The metacercariae of *C. douglasi* are very similar to those of *C. flabelliformis*. It is possible that the metacercariae found in experiments 1 and 2 in a few specimens of *Helisoma* which harbored no cercarial infections were really those of *C. flabelliformis*. Miss Ruby Wortham, who has examined large numbers of *H. trivolvis* from an area where this strigeid is present, informed us that in a few cases small numbers of tetracotyles were found in snails that did not harbor cercarial infections. Since it is impossible to tell from experiments 1 and 2 whether the metacercariae came from natural or experimental infections, the possibility is still present, however, that they were not those of *C. flabelliformis* but of some other closely related strigeid.

It is difficult to understand just why the presence of sporocysts and rediae in physid and planorbid snails makes possible the development of the metacercariae of *C. flabelliformis*. When these abnormal hosts are

<sup>3</sup> A discussion of these early findings is given by Wesenberg-Lund (1934, pp. 141-142).

uninfected with sporocysts or rediae the failure of the metacercariae to develop may be due either to an immune reaction that kills the cercariae after they penetrate or to the absence of suitable conditions for their persistence and development; their death soon after penetration suggests a host reaction but it is possible that suitable food is not available. In the abnormal hosts already harboring cercarial infections the penetrating cercariae of *C. flabelliformis* apparently escape those unfavorable conditions by entering the sporocysts or rediae. Here, as hyperparasites, they find protection from adverse host reactions, entirely escape unfavorable conditions in the tissues, and obtain substances for food that have been prepared by the sporocysts or rediae for their developing progeny. It seems probable, since they develop more rapidly under these conditions than in the tissues of the normal host, that improved nutrition is an important factor in their successful development.

#### SUMMARY

Various species of the family LYMNAEIDAE are the usual second intermediate hosts of the duck strigeid, *Cotylurus flabelliformis*. The cercariae of this strigeid, however, penetrate into planorbid and physid snails, but develop only in those individuals that harbor sporocysts and rediae of larval trematodes; in uninfected snails they do not develop and soon die. The development of the metacercariae in such abnormal hosts takes place inside the sporocysts and rediae where the tetracotyle stage is reached more quickly than in their normal environment which is the hermaphroditic gland of lymnaeid species. It is suggested that when the cercarial bodies penetrate into sporocysts or rediae, they are protected from any immune reaction of the abnormal host, and as hyperparasites, are able to utilize very effectively in their nutrition the food material which these trematode parasites have absorbed from the tissues of the snail host and have prepared for the nourishment of their own developing progeny. Since the development of the metacercariae inside the sporocysts and rediae in the abnormal host appears to be entirely normal, it seems very probable that physid and planorbid snails, which harbor cercarial infections, may have a regular part as second intermediate hosts in carrying out the life cycle of *C. flabelliformis*.

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SPICULE LENGTH IN *COOPERIA CURTICEI* AS A MEASURE  
OF FAVORABLE INTESTINAL ENVIRONMENT FOR  
THIS INTESTINAL NEMATODE OF SHEEP

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From a study of the distribution of trichostrongylids in the small intestine of the sheep Tetley (1937) concluded that their particular location was determined by the rate of response of incoming larvae to conditions within the alimentary canal and that, following taking up their station, virile subsequent stages of the parasites migrated to an inappreciable degree. It was further concluded that parasites were able to survive in a wider range of area and physiological condition of the small intestine than they actually invaded.

In pursuance of this investigation it was considered that comparative study of parasites recovered from different intestinal regions in a given host would add to information on their relation to the environment within the given host. Thus comparison of the extent of their growth in various parts of the intestine by indicating something of the relative suitability of these regions for the parasites would be a step towards understanding how parasites come to select their chosen site of establishment.

Length of spicules has been taken as a suitable index of growth, for the ease with which these structures may be measured with accuracy, and the fact that they are not affected by the various treatments preparatory to measurement, rendered them technically convenient to handle. *Cooperia curticei* was chosen as a favorable species for investigation. Its extended range of infection in the jejunum and the constancy of its frequency distribution made it likely that comparison could be carried out between sections of a population between which there had been a minimum of intermingling.

MATERIAL AND METHODS

Material was collected from infections acquired in the field by Romney cross bred sheep at Palmerston North, New Zealand. Ingesta containing worms were removed from sections of the small intestine with as little disturbance of those adjoining as possible. Worms were preserved in glycerin-alcohol. Measurements were made of glycerin jelly mounts using an ocular micrometer.

RESULTS

The results pertaining to three sheep are contained in Tables 1, 2, and 3. For convenience of tabulation the data have been grouped in 5

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TABLE 1.—*The spicule measurements of Cooperia curticei from three sections of the small intestine as measured from the anterior end (Sheep I)*

Class (microns)	Frequencies of spicule lengths, in micron classes, for intestinal sections:			
	10-15 feet	16-20 feet	21-25 feet	Total, 10-25 feet
111-115 .....	2	—	1	3
116-120 .....	30	6	16	52
121-125 .....	45	—	21	66
126-130 .....	76	8	33	117
131-135 .....	65	15	29	109
136-140 .....	26	2	19	47
141-145 .....	14	—	7	22
146-150 .....	2	1	2	4
Total spicules measured	260	32	128	420
Mean length $\pm$ P.E. ....	128.7 $\pm$ 0.3	129.5 $\pm$ 0.8	129.0 $\pm$ 0.4	128.9 $\pm$ 0.2
Standard deviation .....	6.9	6.7	7.1	6.9

TABLE 2.—*The spicule measurements of Cooperia curticei from five sections of the small intestine as measured from the anterior end (Sheep II)*

Class (microns)	Frequencies of spicule lengths, in micron classes, for intestinal sections:					
	1-5 feet	6-10 feet	11-15 feet	16-20 feet	21-25 feet	Total, 1-25 feet
121-125	—	4	2	7	6	19
126-130	3	14	2	13	12	44
131-135	19	34	2	19	22	96
136-140	5	16	8	16	18	63
141-145	4	11	10	6	6	37
146-150	3	3	2	5	—	13
151-155	—	—	—	—	4	4
156-160	—	—	—	2	2	4
161-165	—	—	2	—	—	2
Total spicules measured	34	82	28	68	70	282
Mean length $\pm$ P.E. ....	135.7 $\pm$ 0.6	133.9 $\pm$ 0.4	139.5 $\pm$ 1.1	134.2 $\pm$ 0.6	135.0 $\pm$ 0.4	134.9 $\pm$ 0.3
Standard deviation	5.1	5.8	8.4	7.7	5.5	7.6

TABLE 3.—*The spicule measurements of Cooperia curticei from five sections of the small intestine as measured from the anterior end (Sheep III)*

Class	Frequencies of spicule lengths, in micron classes, for intestinal sections:					
	8-10 feet	11-12 feet	13-14 feet	17-18 feet	23-24 feet	Total, 8-24 feet
121-125	—	3	2	—	1	8
126-130	2	3	3	2	5	15
131-135	16	6	25	5	15	67
136-140	10	5	19	5	11	50
141-145	5	4	9	2	13	33
146-150	1	3	2	—	1	7
151-155	—	—	—	—	2	2
Total spicules measured	34	24	60	16	48	182
Mean length $\pm$ P.E. ....	135.5 $\pm$ 0.5	135.3 $\pm$ 1.0	135.7 $\pm$ 0.4	133.7 $\pm$ 1.0	136.5 $\pm$ 0.6	135.6 $\pm$ 0.3
Standard deviation	4.6	7.4	4.8	5.9	6.4	5.8

micron classes. Computations have been made, however, on the basis of one micron classes in which form the data had been originally collected.

#### DISCUSSION

The material having been obtained from infection acquired in the field, it is not possible to determine precisely the factors, specific and non-specific, that might have operated to cause particular portions of the worm population to inhabit particular portions of the small intestine. This would be difficult were the material from pure infections of one age. It is concluded that the normal frequency distribution found in the data for spicule lengths for populations within the various regions of intestinal residence is indicative of the uniformity of the growth conditions for the majority of worms within those particular areas. If there had been a selective action, by factors imposed on the worms in the course of their establishment in those regions of the intestine which they were found to be inhabiting, it would be expected that some distinctive form of distribution would have resulted and would have been recognizable. This assumption underlies the possibility that comparison of spicule lengths of the sub-populations of the various areas would indicate the relative favorability of the growth conditions for spicules within those areas. The fact that differences as between different sheep (see below) are thus recognizable lends support to the assumption, that differences within a given sheep would similarly be apparent.

From Tables 1, 2 and 3 it can be seen that the means for spicule length of *C. curticei* for the different intestinal regions in which the parasite established itself, actually exhibited variance to only an insignificant degree. Between sheep I and, on the other hand, sheep II and III the means for the total numbers of spicules measured, differed materially, and to a statistically significant degree. The conclusion is therefore drawn that the sum of the effects of the various influencing factors differed to a greater extent between sheep than between regions of residence in the same sheep. This is another way of saying that from the point of view of growth to maturity the regions for which data were available displayed no significant differences in favorability.

#### SUMMARY

In material from field-acquired infections comparison was made of the lengths of spicules of *Cooperia curticei* found in different parts of the small intestine of sheep. While inter-host differences in the mean lengths of spicules were statistically demonstrable, intra-host differences as between different levels of intestinal residence, were not apparent. It is concluded that in regard to favorability for growth all parts of the whole intestinal area in which the worms established themselves, and

from which they were selectively measured for spicule length, were equally acceptable to this nematode parasite.

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HAEMONCHUS CONTORTUS EGGS: COMPARISON OF  
THOSE IN UTERO WITH THOSE RECOVERED  
FROM FECES, AND A STATISTICAL METHOD  
FOR IDENTIFYING *H. CONTORTUS* EGGS  
IN MIXED INFECTIONS

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By confining attention to the cores of the frequency distribution of lengths plotted against widths, the author (1935) found that it was possible to distinguish between the greater number of eggs of *Nematodirus flicollis* and *N. spathiger*. Shorb (1939, 1940) has applied the method to eggs dissected from worms of other species of trichostrongylids found in ruminants.

For the reason that normal practice requires identification of eggs voided in feces it seemed desirable that comparison should be made between eggs dissected from females and those which had been recovered from feces by ordinary isolation techniques. Tetley (1941) found that among species of *Nematodirus* eggs obtained from feces and measured in the course of making dilution counts by the Stoll method no change in form from that of eggs freshly dissected from females could be detected. In the present account the, by contrast, thin-shelled eggs of *Haemonchus contortus* are considered.

METHODS AND MATERIALS

An ocular micrometer was used in measuring eggs. Dissections of eggs from females and measurements were carried out on fresh material, within a few hours of the death of the host. Eggs of fecal origin were measured partly in the course of making dilution counts by the Stoll method and on other occasions following isolation by a centrifugal method that involved flotation in saturated salt solution.

With the exception of the data from sheep VI and VII of a Dorset-Shropshire cross, located at the Rockefeller Institute for Medical Research, Princeton, New Jersey, the bulk of the data was derived from Romney cross sheep at Palmerston North, New Zealand.

*Sheep I:* Slaughtering date: May 20, 1937. Age: Eight to nine months. Ninety-seven segmented eggs were dissected from 8 females of *H. contortus* and measured. The results are contained in Table 1. Eggs

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were also dissected from females of a number of other species present. The range of distribution of all species has been plotted in Fig. 1.

*Sheep II*: Slaughtering date: March 11, 1936. Age: Seven to eight months. One hundred and eighty-four segmented eggs were dissected from 4 worms taken at random, and measured. Results are contained in Table 1.

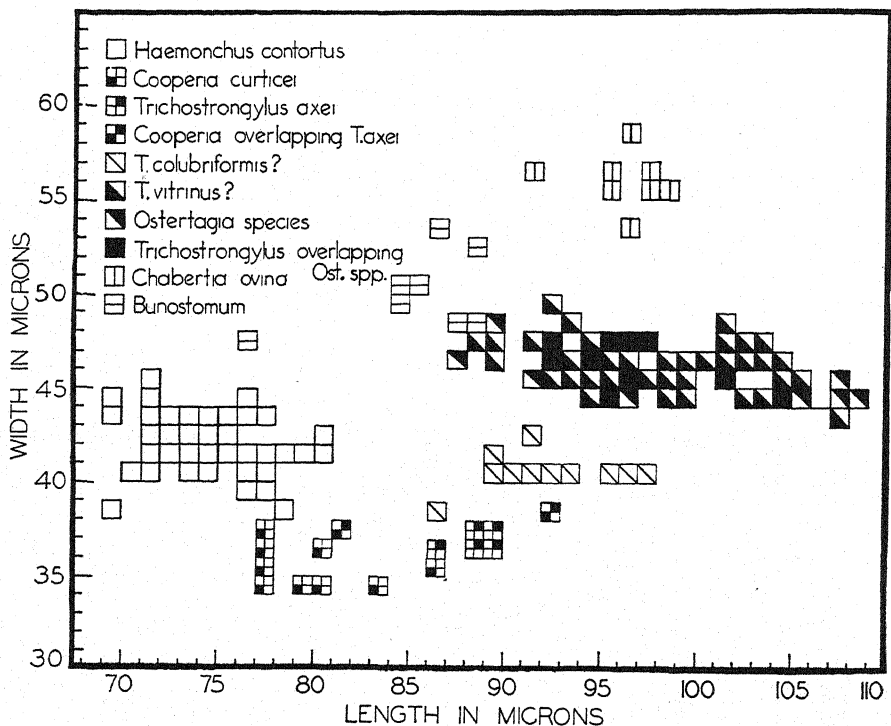


FIG. 1. Range of measurements of eggs dissected from *Haemonchus contortus*, *Cooperia curticei*, *Trichostrongylus axei*, *T. colubriformis* (?), *T. vitrinus* (?), *Ostertagia* species, *Chabertia ovina* and *Bunostomum trigonocephalum* from sheep. For details of the *Haemonchus* data see Table 1. The relative disposition of the specific groupings approximates to that found among eggs from feces in mixed infections (Fig. 8).

*Sheep III*: Slaughtering date: March 16, 1936. Age: Seven to eight months. Two hundred and thirty-four segmented eggs were dissected from 8 worms selected at random, and measured. Results are contained in Table 1.

*Sheep IV*: Slaughtering date: March 5, 1932. Age: Seven to eight months. From 4 females of *Haemonchus contortus* taken at random, 77 segmented eggs were dissected and measured. The results are contained in Table 1 and Fig. 2.

*Sheep V*: Slaughtering date: May 26, 1935. Age: Five years. At

TABLE 1.—Statistical analysis of data on the dimensions of eggs of *Haemonchus contortus*. For method of computation of range of fractions of the egg populations see text

Sheep	Source of eggs : worm	Number of eggs measured	Mean		Size range of eggs		
			Length $\mu$	Width $\mu$	100 per cent (observed) $\mu$	91.2 per cent (computed) $\mu$	25 per cent (computed) $\mu$
I	A	14	76.2	42.9	67-81 by 38-45	70-80 by 38-45	73-77 by 40-42
	B	30	75.0	41.1			
	C	22	78.1	39.4			
	D	11	70.3	43.1			
	Total A-D	77	75.12 $\pm$ 0.19	41.28 $\pm$ 0.14			
			S.D. 2.44 C.V. 3.3%	S.D. 1.78 C.V. 4.3%			
II	E	60	78.9	40.3	66-87 by 38-44	70-83 by 38-42	74-79 by 39-41
	F	45	74.4	40.9			
	G	34	74.3	40.0			
	H	45	77.7	39.7			
	Total E-H	184	76.68 $\pm$ 0.16	40.21 $\pm$ 0.06			
			S.D. 3.29 C.V. 4.3%	S.D. 1.13 C.V. 2.8%			
III	I	55	74.6	42.7	69-86 by 39-46	70-84 by 39-44	74-79 by 41-43
	J	45	77.8	40.9			
	K	10	77.8	41.7			
	L	13	73.2	41.2			
	M	16	72.1	41.3			
	N	15	76.3	40.8			
	O	40	79.4	41.6			
	P	40	79.5	41.4			
	Total I-P	234	76.84 $\pm$ 0.16	41.58 $\pm$ 0.06			
			S.D. 3.57 C.V. 4.6%	S.D. 1.37 C.V. 3.3%			
IV	Q	14	78.0	42.4	70-81 by 39-47	69-80 by 39-45	73-76 by 41-43
	R	17	75.0	42.1			
	S	15	72.6	41.8			
	T	12	77.5	43.9			
	U	10	76.6	40.4			
	V	9	75.3	40.4			
	W	10	72.8	41.2			
	X	10	71.6	44.1			
	Total Q-X	97	74.54 $\pm$ 0.18	42.09 $\pm$ 0.11			
			S.D. 2.62 C.V. 3.5%	S.D. 1.51 C.V. 3.6%			
V	Y	66	77.7	45.3	63-93 by 31-48	72-85 by 38-46	76-80 by 41-43
	Z	30	78.1	41.8			
	AA	25	78.9	34.6			
	BB	30	80.1	42.5			
	Total Y-BP	151	78.36 $\pm$ 0.19	41.92 $\pm$ 0.11			
			S.D. 3.13 C.V. 4.0%	S.D. 1.84 C.V. 4.4%			
I-V	Total 28 worms	743	76.61 $\pm$ 0.09	41.25 $\pm$ 0.05	66-87 by 34-47	69-84 by 37-46	74-79 by 40-43
			S.D. 3.78 C.V. 4.9%	2.20 5.3%			
			S.D. 3.11 C.V. 4.0%	S.D. 1.72 C.V. 3.8%			
V	Feces	111	78.49 $\pm$ 0.19	44.90 $\pm$ 0.11	72-89 by 39-51	72-85 by 41-48	76-81 by 44-46
			S.D. 3.69 C.V. 5.2%	S.D. 1.66 C.V. 3.7%			
VI	Feces	60	70.77 $\pm$ 0.32	45.15 $\pm$ 0.14	62-80 by 42-50	63-78 by 42-48	68-73 by 44-46
			S.D. 3.82 C.V. 5.2%	S.D. 1.94 C.V. 4.3%			
VII	Feces	60	73.72 $\pm$ 0.33	45.48 $\pm$ 0.17	65-83 by 42-50	66-81 by 42-49	71-76 by 44-47
			S.D. 3.82 C.V. 5.2%	S.D. 1.94 C.V. 4.3%			

S.D. = Standard deviation.  
C.V. = Coefficient of variation.

autopsy 750 females of *H. contortus* and a few individuals of *Ostertagia circumcincta*, *O. trifurcata* and *Trichostrongylus axei* were found. One hundred and twenty-seven eggs were broken out from 4 females of *H. contortus* taken at random, and measured. These results are summarized in Table 1. Measurements of eggs found in feces in the course of making dilution counts by the Stoll method were made at intervals over a period of approximately three years. The aggregated results from this latter source are plotted in Fig. 4. Measurements of fecal eggs made at the time of autopsy have been replotted in Fig. 3.

*Sheep VI and VII*: Date of fecal sampling: May 7, 1940. Age of sheep: Over one year. Pure infections of *H. contortus* were present in these two animals. Eggs were secured from freshly collected feces by means of a centrifugal flotation method, using saturated salt solution. Measurements were made of 60 eggs from each animal and these are plotted in Figs. 5 and 6 respectively.

*Flock of fifty sheep*: Dates of fecal sampling: Between March 7, 1935, and January 21, 1936. Age of sheep: Five years and older. Measurements of eggs were obtained in the course of making counts by the Stoll dilution technique of eggs in periodically collected samples of feces from a flock of 50 sheep. These are plotted in Fig. 8.

#### ANALYSIS OF DATA

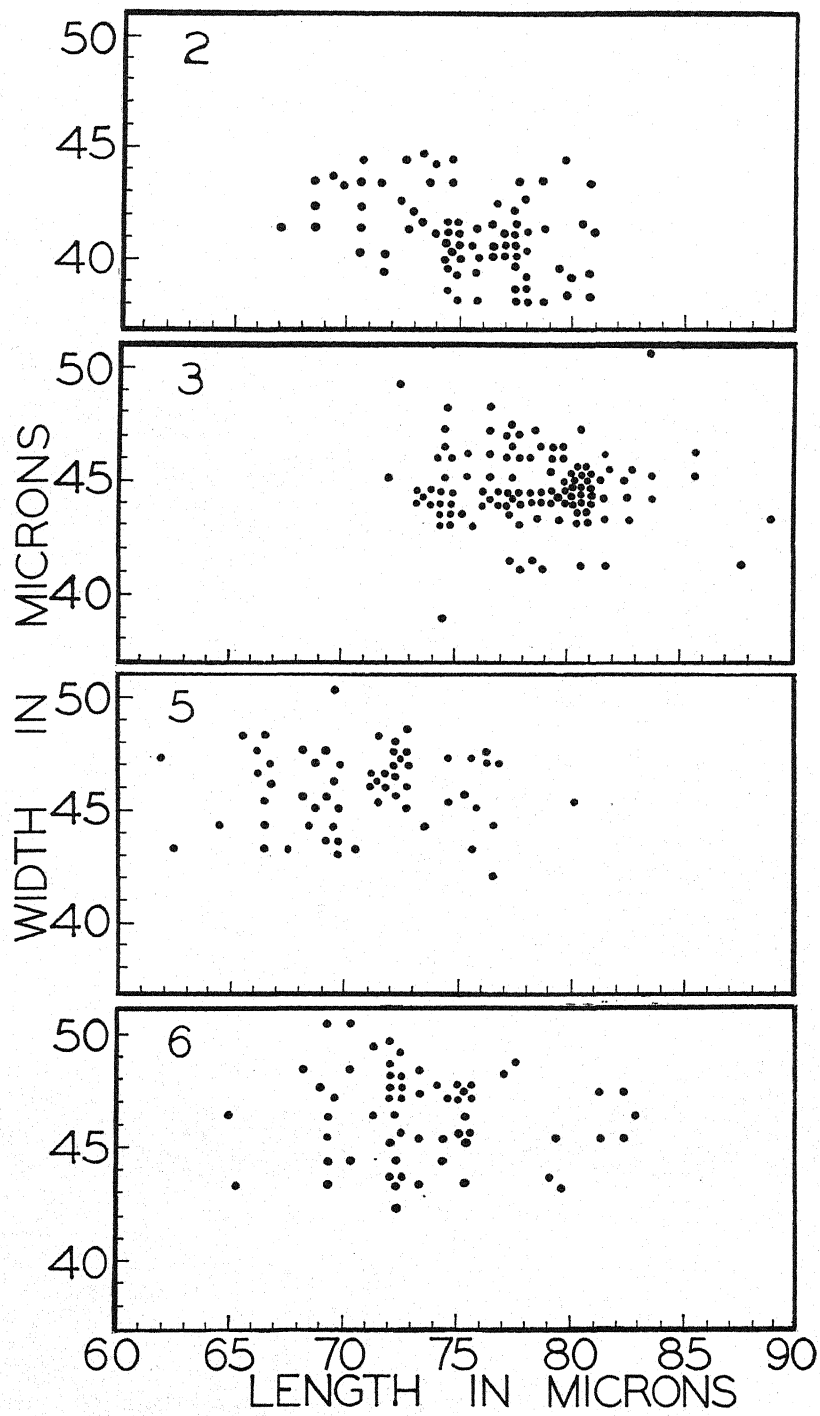
A statistical analysis of the data has been made. This is set out in Table 1. Means for aggregated results were computed from the totals of eggs from sheep, not from the means for individual worms. The populations of eggs have been regarded as having a bivariate normal frequency distribution and accordingly formulae relating to such distributions have been applied to the data. The small amount of negative correlation between the two axes has been ignored. Statistical ranges of means plus and minus respectively 0.6745 and twice the standard deviations which in a universe would enclose respectively 25 per cent and 91.2 per cent of the population have been calculated for the data, to the nearest micron.

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FIG. 2. Length plotted against width of eggs dissected from *Haemonchus contortus* from sheep IV (see Table 1). The bivariate normal frequency distribution is typical for eggs dissected from worms from other sheep (see Table I and Fig. 7). Width of eggs dissected from worms tends to be less than that of eggs obtained from feces (see Figs. 3, 5, and 6).

FIG. 3. Length plotted against width of eggs of *Haemonchus contortus* obtained from feces of sheep V at autopsy (see Table 1). The grouping persisted for eggs from the feces of the same animal over three years (see Fig. 4) and coincided with a group among the measurements of eggs from feces in 50 mixed infections (see Fig. 8).

FIGS. 5 and 6. Length plotted against width of eggs of *Haemonchus contortus* from the feces of sheep VI and VII, respectively. Width is similar to that of eggs from the feces of sheep V (Fig. 3) though length is less.



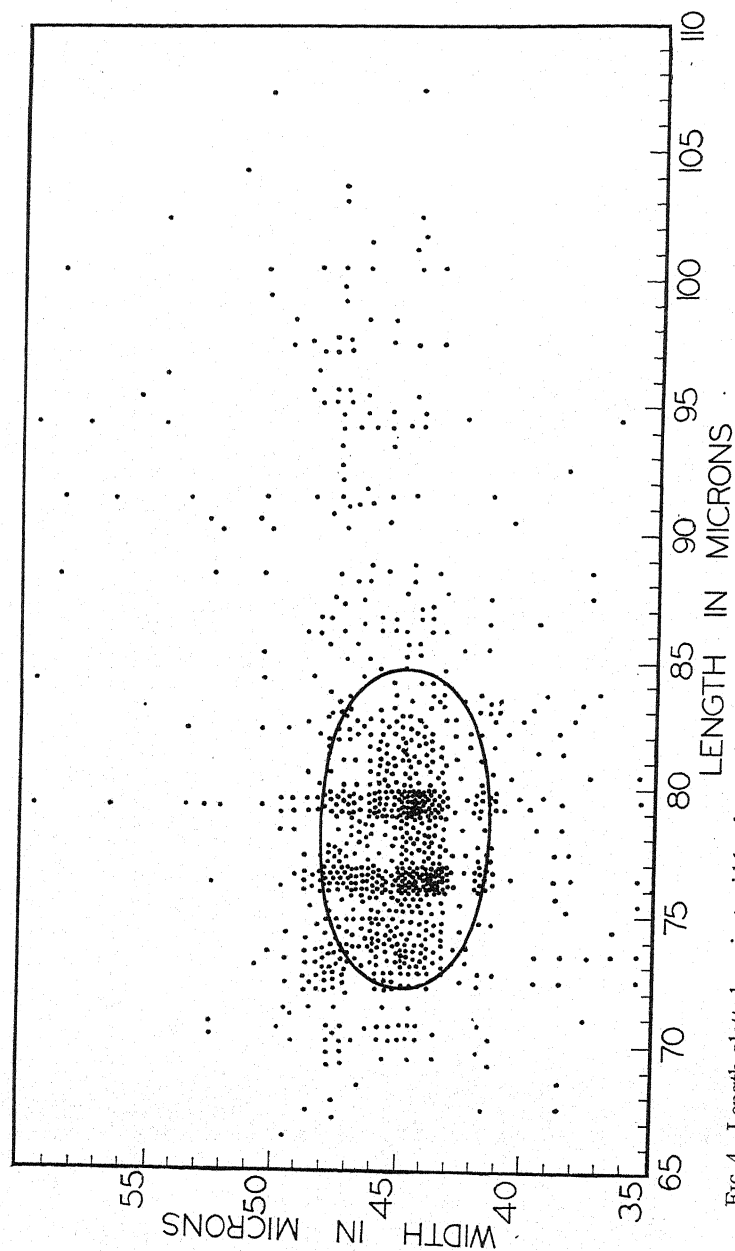


FIG. 4. Length plotted against width of eggs from feces of sheep V over a period of 3 years during the earlier portion of which mixed infection was present. The imposed curve is based on Fig. 2 and represents the statistical range of the mean axial lengths plus and minus twice the standard deviation, this being computed to enclose 91.2% of the egg population.



COMPARISON OF EGGS DISSECTED FROM WORMS WITH THOSE  
OF FECAL ORIGIN

It is concluded that, as *H. contortus* found in sheep V at post mortem considerably outnumbered other species and because the egg-laying rate of the former species is known to exceed that of other trichostrongylids found in sheep, the measurements of eggs from feces obtained from this sheep at the time of autopsy (Fig. 3) may be regarded as differing insignificantly from what might have been derived from a pure infection.

The methods of sampling material for measurement differed between the two series. Eggs of fecal origin were the contributions of many worms which individually were represented in the total in proportion to their fecundity. On the other hand eggs dissected from worms were obtained from a small fraction of the entire population of parasites and further, the numbers measured were not numerically related to the potential egg-laying rate of the individuals dissected.

In regard to the worms used for dissection it is concluded, from the bivariate normal frequency distribution of the means of the axes of eggs from individuals (Fig. 7), that the samples adequately represented the general population of worms.

The similar coefficients of variation for fecal eggs from sheep V and for the aggregated results from dissections, respectively 4.0 per cent and 4.9 per cent for lengths and 3.8 per cent and 5.3 per cent for widths, were indicative that any error arising from disregarding the rate of egg-laying was insufficient to invalidate comparison of the two series of data. It follows that in computing means for dissected-out-eggs there was little difference between values derived from averaging the aggregated data, and the unweighted values from averaging the means for individual worms.

As shown in Table 1 means for the aggregated data for eggs from dissections were found to be  $76.61 \pm 0.09 \mu$  by  $41.25 \pm 0.05 \mu$  and those for fecal eggs from sheep V  $78.49 \pm 0.19 \mu$  by  $44.90 \pm 0.11 \mu$ . Thus it is seen that they reflect the relation between the two series of data shown in the range of fluctuation (Figs. 7 and 3), which involved almost complete overlapping of the long axes but, in regard to the short axes, a lesser degree of overlapping.

A small degree of negative or an absence of correlation was displayed between the long and the short axes (Figs. 3 and 7).

From the foregoing it is evident that the most marked difference between the two series of data was in connection with mean width. This is the dimension which would be expected to be most affected by any change in volume due to increase in internal pressure in eggs. However, what factors were operating between the time of laying of the eggs and the examination of the eggs under the microscope and were instrumental in

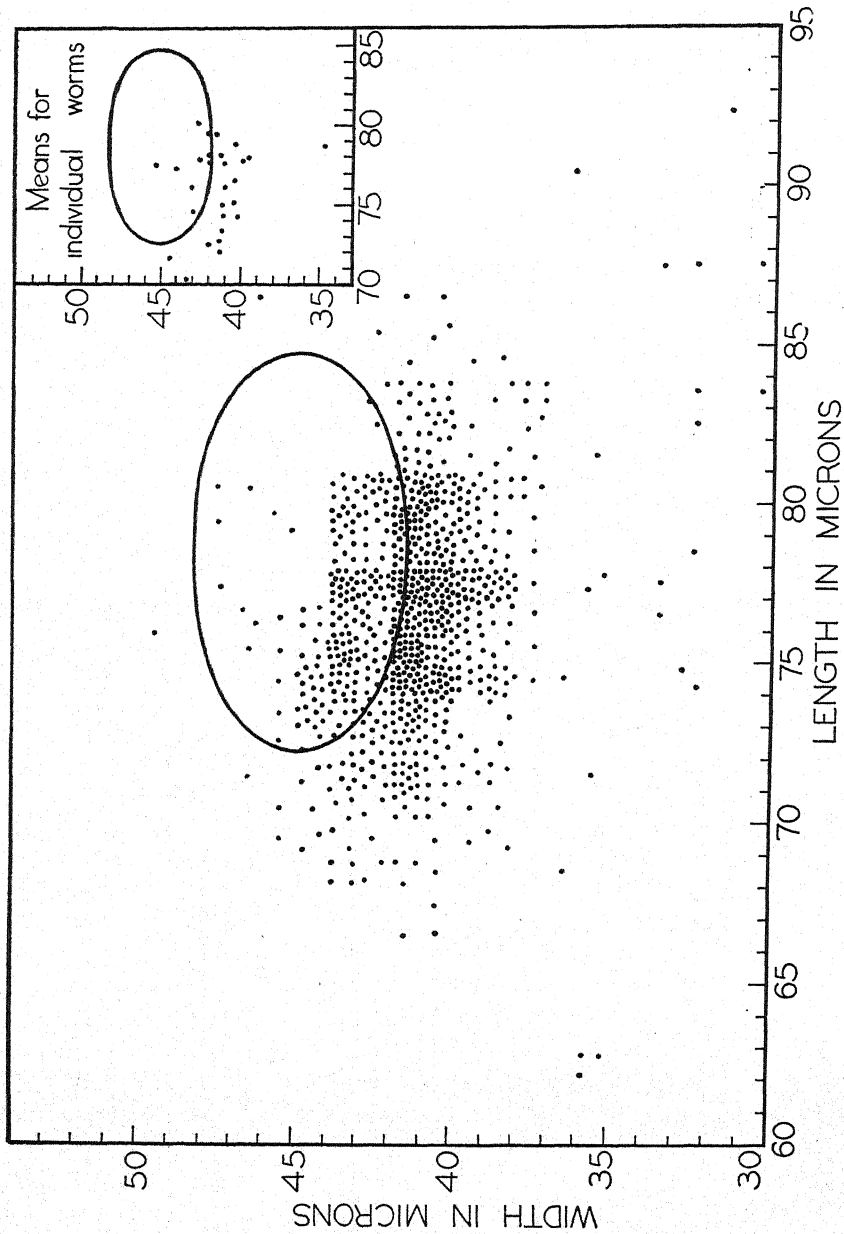


FIG. 7. Comparison of the measurements of eggs of *Haemonchus contortus* obtained by dissection with those from feces, to demonstrate the greater diameter of the latter. Length has been plotted against the width of eggs dissected from worms from sheep I-V (see Table 1). The imposed curve is based on the measurements of eggs from the feces of sheep V (see Table 1 and Fig. 3).

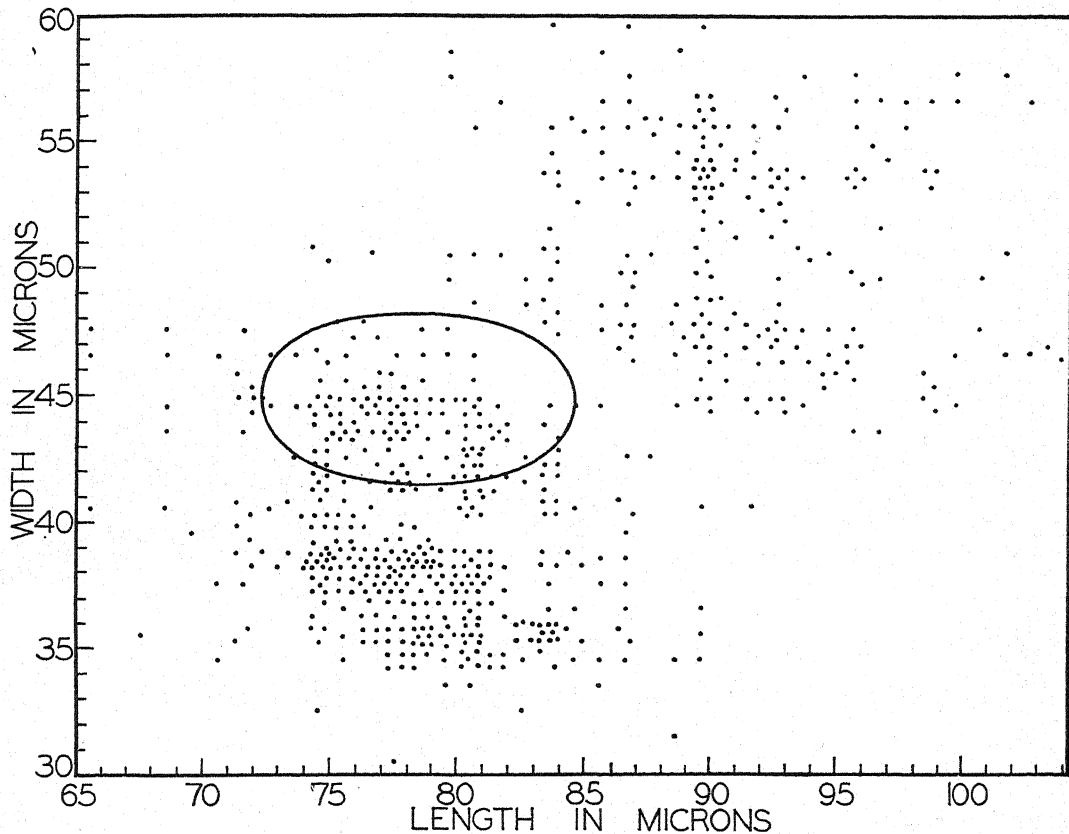


Fig. 8. Showing that a curve based on measurements of *Haemonchus contortus* eggs from the feces of sheep V and computed to enclose 91.2 per cent of the population of eggs fits a group of measurements of eggs from the feces of 50 mixed infections.

causing the change in volume are not indicated by the data. It is possible that the technical methods used were not entirely responsible.

Conclusions as to differences among the data of fecal origin from sheep V, VI and VII cannot be drawn. The latter two sheep, of different breed from sheep V, contained experimentally administered small infections of *H. contortus* and had been maintained indoors; further, another technique, centrifugal flotation, had been used in isolating the eggs from feces; then also there was the possible existence of strains among the parasites and of other factors associated with the geographically widely separated origin of the material; these all, conceivably, might have contributed causally to the differences.

APPLICATION OF RESULTS IN THE IDENTIFICATION OF EGGS  
VOIDED IN FECES

When plotted, the complete data relating to eggs of fecal origin from sheep V and from the flock of 50 sheep fell into groups between which there were various degrees of overlapping (Figs. 4 and 8). In each series a group coincided with that established for *H. contortus* from measurements made on eggs from feces of sheep V collected at the time of autopsy (Fig. 3). The conclusion is that, as the identity of the *H. contortus* grouping was well defined in data collected from the one sheep over a period of three years and from the flock of 50 sheep over several months, the frequency was characteristic of the species.

Applying to the complete data for sheep V (Fig. 4) and to that for the flock of 50 sheep (Fig. 8) a curve, constructed on the basis of measurements of fecal eggs from sheep V made at the time of autopsy and estimated to enclose 91.2 per cent of the population, it is seen that it encircles the core of the *Haemonchus* group in each frequency series. It is concluded that eggs mainly of *H. contortus* were found within the range of the curve.

In the presence of overlapping species it is considered that greater accuracy in estimating the number of *H. contortus* eggs would be achieved by applying curves containing smaller fractions of the population for instance 25 per cent (i.e., means plus and minus 0.6745 the standard deviation), as it is to be expected that towards the center of a distribution the proportional contamination by other species would be reduced. The measurement of large numbers of eggs is an obvious requirement for the successful application of the method.

It is possible that constants for *Haemonchus* will have to be determined for various conditions, inasmuch as host species or breed, strain of parasites and technical methods used might, to varying degrees, influence the form of the eggs; results from sheep VI and VII (Figs. 5 and 6) suggest this.

## VALUE OF MEASUREMENTS OF EGGS DISSECTED FROM WORMS

The results plotted in Fig. 1 are designed to show that inter-specific differences in egg measurements of material from the one sheep were well marked; to this extent they supplement the findings of Shorb (1940) who does not specify the number of animals from which he obtained his data. It is reasonable to expect that parallel differences will exist in the dimensions of eggs found in feces and that, therefore, similar constants might be established for these other species on the lines given above for *H. contortus*. The dimensions of eggs dissected from females may possibly have an intrinsic value when treated statistically from the point of view

of their use in the specific identification of females in certain genera, at present distinguishable only with difficulty.

#### SUMMARY

Measurements of eggs of *Haemonchus contortus* were made in New Zealand, using material derived partly from dissections of females and partly from the feces of sheep. Standard techniques were used.

It was concluded that swelling of eggs took place during the interval between laying and the time of their examination under the microscope.

When lengths of eggs were plotted against widths the population had a bivariate normal frequency distribution.

The statistical range of means plus and minus twice the standard deviation (theoretically enclosing 91.2 per cent of the population) for eggs from feces treated by the Stoll technique was found to be 72–85  $\mu$  by 41–48  $\mu$ . This range fitted a group in data collected by the same technique from mixed infections in one sheep over three years and fifty sheep over several months.

In mixed infections where overlapping in dimensions by other species takes place it is suggested that the smaller range, 76–81  $\mu$  by 44–46  $\mu$  (means plus and minus 0.6745 the standard deviation and theoretically enclosing 25 per cent of the population), would enable the more accurate estimation of the numbers of *Haemonchus* eggs present. It is possible that the constants will be dependent on strain of parasites, technique used and host species or breed.

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## RESEARCH NOTES

### A CASE OF URINARY MYIASIS

A female patient, age 32, reported to Dr. S. J. Ohlhausen, of Houston, Texas, that she noticed about a dozen maggots in a toilet bowl after urination. Uncertain whether the maggots were actually voided with the urine or had gotten into the toilet bowl from some other source, she urinated further into a clean pan and found three more living maggots, which she brought to Dr. Ohlhausen, who, after killing and preserving them in formalin, referred them to the writer for identification. Examination showed them to be third-stage larvae of a *Lucilia*. The size and spacing of the posterior stigmal plates and the eight lobes of the anterior spiracles suggests that it is either *Lucilia sericata* or a closely related species.

The patient complained of pain in the bladder region and a burning sensation upon urination, which continued for several days after the worms were passed, although no more were found. The urinalysis on the day the worms were passed showed the following features: appearance, cloudy; reaction, alkaline; leucocytes, ++++; erythrocytes, ++; epithelial cells, occasional; albumin, ++++; and a heavy, mixed flora of motile bacilli. A urinalysis made two days after passage of the worms still showed abundant leucocytes and erythrocytes. Examination of the vulva and vagina showed no abnormal conditions. The patient offered no information which would indicate the source of the infection.—ASA C. CHANDLER, *Rice Institute, Houston, Texas.*

### EXAMINATIONS OF WILD ANIMALS FOR THE CATTLE TICK *BOOPHILUS ANNULATUS MICROPLUS* (CAN.) IN FLORIDA

The recent attempt to free Florida of the North American cattle tick, *Boophilus annulatus* (Say), and its southern variety, *B. a. microplus* (Can.), by reducing the deer population in certain sections where this tick had not yielded to the usual eradication procedure, offered an opportunity to make a collection of wild hosts in the remaining "ticky" areas of the state. The majority of the collections were made during the months of November, December, January, and February, 1936-37, in collaboration with the U. S. Bureau of Animal Industry and the Florida State Livestock Board. A few records are included that were made in June, 1933, by O. C. Van Hyning. The collections were from Orange, Osceola, and Collier Counties, where the cattle ticks were known to occur. Since the host-parasite relationships are well established for domestic animals, the few records for such animals are included here only to give data on species other than cattle ticks. The records for wild birds and animals that follow include fairly representative numbers of the principal forms in the region. Although the number of hosts examined in this survey is not large, it is thought that the records should be published because of the present active interest in this question. These records are in accord with experimental data gathered by research units and with observations made by many men working on the tick problem. They sustain the conclusion that wild birds and animals other than deer do not serve as hosts for cattle fever ticks. In Table 1 are listed the 16 hosts with the 9 species of ticks collected. Following the table is a list of animals examined that harbored no ticks. The numbers in parentheses placed after the name of the hosts that were negative indicate the number of specimens examined.

Of the 22 deer examined (all from Orange County), 4, which were killed in a large cypress swamp, were infested with cattle ticks, the average infestation being 69, with a range from 3 to 182. The 18 deer on which there were no cattle ticks were taken from pine flat woods. This absence of cattle ticks on deer taken from the woods appears to be correlated with a program of ranging cattle over the tick-infested areas and dipping them at regular intervals. In the swampy areas it has not been possible to control ticks with such a cattle-dipping program, because the cattle do not move about freely in the dense swamps frequented by deer.

TABLE 1.—Species of ticks collected from wild and domesticated animals in the cattle tick areas of Florida

Hosts			Species of ticks								
	Number of specimens examined	Number infested with ticks	<i>Haemophysalis leporis palustris</i> Pack.	<i>H. chordalis</i> Pack.	<i>Amblyomma maculatum</i> Koch	<i>A. americanum</i> (L.)	<i>Dermacentor variabilis</i> (Say)	<i>Ixodes texanus</i> Banks	<i>I. ricinus</i> scapularis Say	<i>Rhipicephalus sanguineus</i> (Latr.)	<i>Boophilus annulatus microplus</i> (Can.)
BIRDS											
Red-bellied woodpecker, <i>Centurus carolinus</i> (L.) ...	1	1			+						
Southern meadowlark, <i>Sturnella magna argutula</i> Bangs .....	55	42	+	+							
White-eyed towhee, <i>Pipilo erythrophthalmus</i> <i>alleni</i> Coues .....	1	1	+	+							
Florida grasshopper sparrow, <i>Ammodramus savannarum</i> <i>floridanus</i> (Mearns) .....	1	1			+						
MAMMALS											
Florida opossum, <i>Didelphis virginiana pigra</i> Bangs .....	4	3					+		+		
Florida raccoon, <i>Procyon lotor elucus</i> Bangs.	43	16					+	+	+		
Florida gray fox, <i>Urocyon cinereoargenteus</i> <i>floridanus</i> Rhoads .....	1	1			+				+		
Florida bobcat, <i>Lynx rufus floridanus</i> (Raf.)	2	2							+		
Florida cotton mouse, <i>Peromyscus gossypinus</i> <i>palmarius</i> Bangs .....	22	15					+				
Florida cotton rat, <i>Sigmodon hispidus littoralis</i> Chapman .....	25	21					+				
Florida cottontail, <i>Sylvilagus floridanus flori-</i> <i>danus</i> (Allen) .....	1	1	+								
Florida marsh rabbit, <i>S. palustris paludicola</i> (Miller and Bangs) .....	4	3	+								
Florida deer, <i>Odocoileus osceola</i> (Bangs).	22	13			+				+		+
Hog .....	10	9			+		+		+		
Dog .....	9	4					+		+	+	
Man .....	?	3			+	+		+			

The following mammals were examined and found to have no ticks on them: Florida skunk, *Mephitis elongata* (Bangs) (3), Southern gray squirrel, *Sciurus carolinensis carolinensis* Gmelin (1), Southern fox squirrel, *S. niger niger* L. (2), and horse (4).

The following birds were examined and found to have no ticks on them: turkey vulture, *Cathartes aura septentrionalis* Wied. (2); Florida red-shouldered hawk, *Buteo lineatus alleni* Ridg. (2); Florida bob-white, *Colinus virginianus floridanus* (Coues) (2); Florida turkey, *Meleagris gallopavo osceola* Scott (2); killdeer, *Oryxechus vociferus vociferus* (L.) (1); Eastern mourning dove, *Zenaidura macroura carolinensis* (L.) (2); Florida barred owl, *Strix varia alleni* Ridg. (1); Florida burrowing owl, *Speotyto cunicularia floridana* Ridg. (2); chuck-will's widow, *Antrostomus carolinensis* (Gmel.) (1); red-cockaded woodpecker, *Dryobates borealis* (Vieill.) (2); crow, *Corvus brachyrhynchos brachyrhynchos* Brehm (with some

doubt about the identity of the subspecies) (2); brown-headed nuthatch, *Sitta pusilla pusilla* Lath. (1); mockingbird, *Mimus polyglottos polyglottos* (L.) (1); robin, *Turdus migratorius migratorius* L. (with some doubt about the identity of the subspecies) (2); bluebird, *Sialia sialis grata* Banks (with some doubt about the identity of the subspecies) (3); loggerhead shrike, *Lanius ludovicianus ludovicianus* L. (3); and grackle, *Quiscalus quiscula aglaeus* Baird (with some doubt about the identity of the subspecies).

Identifications of the ticks were by F. C. Bishopp, C. N. Smith, and H. L. Trembley of the Bureau of Entomology and Plant Quarantine.—BERNARD V. TRAVIS, *Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.*

#### A COLLECTION OF OHIO TICKS AND THEIR HOSTS

A collection of ticks in Ohio by the writer, assisted by Dr. Floyd B. Chapman, yielded the following information. There are forty records, including six records of retrapped animals. The hosts were seven mammalian species, three avian species, and four records of ticks collected from vegetation by a flagging method. Collection was made during September, October, and November in 1936, and April through October in 1937. Three genera of ticks are recognized, including five species: *Haemaphysalis leporis-palustris*, *Dermacentor variabilis*, *Ixodes cookei*, *Ixodes diversifossus*, *Ixodes marxi*.

##### I. *Haemaphysalis leporis-palustris*.

###### A. Nile Twp., Scioto Co.

1. Hosts—22 rabbits (*Sylvilagus floridanus mearnsi*)
  - (a) adults: May 5, June 28, 30, July 5, 10, 11, 12, ?, 13—all in 1937.
  - (b) nymphs: Sept. 24, 27, Oct. 4, 6, 26, Nov. 21 in 1936; Aug. 2, 1937.
  - (c) larvae: Sept. 27, Oct. 6, 1936; Aug. 7, 1937.
2. Host—1 catbird (*Dumetella carolinensis*)
  - (a) adult: May 5, 1937.
3. Host—1 quail (*Colinus virginianus virginianus*)
  - (a) nymph: Oct. 26, 1936.

###### B. Oxford Twp., Erie Co.

1. Hosts—4 rabbits (*Sylvilagus floridanus mearnsi*)
  - (a) adults: July ?, 1937.
  - (b) nymphs: Aug. 3 and 25, 1937.
  - (c) larvae: Aug. 25, Sept. 21, 1937.
2. Host—2 records collected from vegetation.
  - (a) larvae: Aug. 20 and 28, 1937.

###### C. Huron Co. (northern part).

1. Host—1 starling (*Sturnus vulgaris vulgaris*)
  - (a) larva: Aug. 10, 1937.

###### D. Licking Co. (Linville, O.).

1. Host—1 rabbit (*Sylvilagus floridanus mearnsi*)
  - (a) nymphs: Sept. 18, 1937.

##### II. *Dermacentor variabilis*.

###### A. Nile Twp., Scioto Co.

1. Host—1 man
  - (a) adult: 1 record between July and August, 1937.

##### III. *Ixodes cookei*.

###### A. Oxford Twp., Erie Co.

1. Hosts—2 woodchucks (*Marmota monax*)
  - (a) adults: Aug. 19, 28 in 1937.
  - (b) nymphs: Aug. 19, 28 in 1937.
  - (c) larvae: Aug. 19, 1937.
2. Host—1 opossum (*Didelphis virginiana*)
  - (a) nymphs: Sept. 4, 1937.

3. Host—1 weasel (*Mustela noveboracensis*)  
(a) adults, nymphs, and larvae: all on Sept. 20, 1937.
  4. Host—1 record collected from vegetation  
(a) nymph: Aug. 3, 1937 (identification not definitely settled).
- IV. *Ixodes diversifossus*.
- A. Nile Twp., Scioto Co.
    1. Hosts—3 rabbits (*Sylvilagus floridanus mearnsi*)  
(a) adults: Apr. 27, July 5 and 10, 1937.
  - B. Oxford Twp., Erie Co.
    1. Host—1 rabbit (*Sylvilagus floridanus mearnsi*)  
(a) larvae: Aug. 25, 1937.
    2. Host—1 record collected from vegetation.  
(a) nymph: July ?, 1937 (identification not definitely settled).
- V. *Ixodes marxi*.
- A. Nile Twp., Scioto Co.
    1. Host—1 gray squirrel (*Sciurus carolinensis carolinensis*)  
(a) adult: Oct. 3, 1936.
  - B. Ashtabula Co.
    1. Host—1 red squirrel (*Sciurus hudsonicus*)  
(a) adult: Sept. 16, 1937.

*Haemaphysalis leporis-palustris* and *I. diversifossus* were found associated on two rabbits in Scioto Co., and one rabbit in Erie Co. *I. cookei* was recovered in all three developmental stages from one woodchuck and one weasel in Erie Co.

This work was performed while a research fellow under Dr. L. E. Hicks at the Ohio Wildlife Research Station, Columbus, Ohio, 1936–1938. Tick species were determined by Dr. F. C. Bishopp and Miss H. L. Trembley of the Department of Agriculture, Washington, D. C.—JULIUS S. KATZ, Junior Veterinarian, U. S. Bureau of Animal Industry.

#### PTERODRILUS ALCICORNUS IN VIRGINIA

In 1895 Moore described a curious branchiobdellid from John's River, Watauga Co., North Carolina. This species he named *Pterodrilus alcicornus* and made it the type of his new genus, *Pterodrilus*. He named the species *alcicornus* from the dorsal appendages, "Their shape being strongly suggestive of the antlers of a young moose."

Since this original specimen described by Moore, no more have been reported. Although the writer had extensive collections from the Eastern mountains during a recent survey of this group, no individual of this species was encountered; so it had to be considered a rare species known only from the type material.

Recently in a collection of worms from Giles Co., Virginia, the writer was astonished to see two specimens of *Pterodrilus alcicornus*. These agreed in every way with Moore's original descriptions. The specimens were sent by Dr. Horton H. Hobbs, Jr., of the University of Florida. They were taken from the crayfish, *Cambarus bartoni*, on August 13, 1940. The infected crayfish were collected in a rocky pool of a small mountain creek known locally as Sinking Creek near Mt. Lake, Giles Co., Virginia.

Giles Co., Virginia, is about 120 miles north of the type locality, and is in the same type of country. *Pterodrilus alcicornus* is probably a species of a very local distribution confined to a small area of the Eastern mountains. While many species of branchiobdellids are wide ranging, some, especially mountain forms of the genus *Pterodrilus*, may be very restricted. This is, of course, the first record of this branchiobdellid from the state of Virginia.—CLARENCE J. GOODNIGHT, Biology Department, Brooklyn College.



## IN MEMORIAM

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ADOLPHO LUTZ (1855-1940)

The death of Dr. Adolpho Lutz on October 6, 1940, terminated the career of one of the ablest and most distinguished scientists of the past generation. He was born in 1855 at Rio de Janeiro, of Swiss parents who came to Brazil in 1849. His father's family had lived in Berne for more than five hundred years and his mother's family resided in a neighboring canton. Adolpho Lutz was taken to Switzerland at the age of two and educated there. He graduated in medicine at the University of Berne in 1879 and was interne at the Hôpital de St. Gallen. He continued his training at Leipzig (with Leuckart), Prague, Vienna, London (with Lister), and Paris (with Pasteur).

With this admirable preparation, he returned to Brazil in 1881 and began the practice of medicine in the interior of the state of São Paulo. His training with the European masters, however, had stimulated a deep interest in research and his work as a physician was augmented by investigations of hookworm and other parasites, chiefly helminths. In 1888 he returned to Europe, where he worked in the dermatological institute of Prof. Unna in Hamburg. He was then invited to direct the work on leprosy in Hawaii (1889-1891) where he met and married an Englishwoman, Amy Fowler, who had gone there as a volunteer nurse for the lepers. From Hawaii, Dr. Lutz went to California where he secured a license to practice medicine. But he soon (1892) returned to Brazil where he reestablished practice and became a member of the staff of the newly organized Bacteriological Institute of São Paulo. Professor Le Dantec had been brought from Paris as the first Director, but his French associate died of yellow fever and Le Dantec was so disturbed and distressed that he resigned his position, recommended the appointment of Dr. Lutz as his successor, and returned to France. Dr. Lutz served as Director of the Bacteriological Institute until September, 1908, when at the invitation of Oswaldo Cruz he went to Rio as Director of the Section of Medical Zoology in the Instituto de Manguinhos, later the Instituto Oswaldo Cruz, which offered more time and opportunity for investigation. He had become more interested in research than in medical practice and found administrative routine an irksome task. He remained at the Instituto Oswaldo Cruz until 1938, although he had been officially retired two years earlier, at the age of eighty-one, when a regulation was adopted retiring all civil servants more than sixty-eight years old.

Like other physicians who were also great parasitologists, notably Leidy, Leuckart and Fülleborn, the principal factor in Adolpho Lutz's

work and career is to be found in his devotion to natural history. His absorbing interest in fundamental biological phenomena was the well-spring from which arose the extensive list of his discoveries in biology and medicine. As a boy of five, he was studying animals and plants. His first paper, published in 1878 and awarded a prize by the naturforschenden Gesellschaft in Berne, was on the Cladocera of the Bernese region. On his first return to Brazil he began his researches in parasitology, especially on hookworm, other endoparasites, and the blood-sucking Diptera. These investigations were continued on his subsequent return and in addition he extended the work to include the bacteria, fungi which attack the skin, and the parasitic protozoa. Endowed with an inquiring mind, he turned his attention to all kinds of disease-producing organisms. His patience and thoroughness are apparent in the results. Each new discovery was the starting-point for further investigation. His studies on schistosomiasis led in 1921 to the disclosure that certain fork-tailed cercariae undergo metamorphosis in intermediate hosts to produce the tetracotyliform larvae, thus correcting the idea of Leuckart that strigeid trematodes have a "metastatic" type of development. This discovery was confirmed almost immediately by Mathias in France and Szidat in Germany, and has provided the basis for the great advance in knowledge of the furcocercous cercariae and the STRIGEIDAE. His investigations of trematode life histories were supplemented by studies on the snails which serve as intermediate hosts. His work on the parasites of amphibians led to the beautifully illustrated monograph on the batrachians of Brazil. From his early years he conducted researches on the blood-sucking arthropods, which culminated in many shorter articles and monographs of several families of Diptera. Familiar with the life histories, habits and distribution of the Culicidae of Brazil, he was able to indict the *Aedes* (*Stegomyia*) as the transmitting agent of yellow fever and he instituted the antimosquito campaign in São Paulo immediately after the results of the Havana experiment were communicated to him by letter. To confirm the work, he raised mosquitos and repeated the experiment on himself. In recognition, the state of São Paulo struck a gold medal in his honor. He published on the blood-inhabiting Sporozoa of lower vertebrates, Microsporidia, trypanosome infection in cattle, and a host of other subjects, in addition to the reports of the laboratory of which he was the Director. The study of parasites and parasitic infections in lower animals afforded the primary information for his discoveries in human medicine.

Dr. Lutz was an enthusiastic collector. He assembled some 3000 herbarium specimens in addition to his extensive helminthological, entomological and herpetological collections. These specimens, together with his letters and other papers, are housed in the Instituto Adolpho Lutz at São Paulo, dedicated three weeks after his death.

The medical contributions of Dr. Lutz are too extensive and too well known to require enumeration here. He introduced vaccination in South America, instituted isolation and quarantine against smallpox, typhoid and yellow fever, and through his vigorous public health measures eliminated yellow fever, bubonic plague and cholera from São Paulo. His more important achievements include: (1) the identification of the "febres paulistas" of São Paulo as typhoid, a determination which was confirmed on cultures by the Institut Pasteur and on anatomical findings by Eberth; (2) the discovery before 1903 of epidemics of yellow fever in forested areas where he knew there were no "*Stegomyia*," but other closely related species; (3) the demonstration (1903) of the transmission of malaria by jungle mosquitos, *Anopheles lutzi* Theobald, which breed in the leaf cups of bromeliad plants; (4) the discovery of granulation in the acid-fast bacilli of leprosy and the proposal (1886) of the name *Coccothrix leprae* for this organism. The study of leprosy for more than fifty years, and especially consideration of its epidemiology, persuaded him that the disease could be transmitted by mosquitos.

Dr. Lutz traveled widely in Europe and the Americas, and was a member of many scientific societies. An accomplished linguist, he was an official delegate on many scientific and medical missions and received numerous medals and decorations from South American and European governments. Brief accounts of his work were published in North America on the occasion of his seventieth birthday by Metcalf (1926, *Scientific Monthly*, 22: 112-114) and after his death by Helen Gaige (1940, *Copeia*, No. 4, pp. 275-276). The *Memorias do Instituto Oswaldo Cruz*, Vol. 18 (1925) contains an intimate article by Dr. Carlos Chagas and a bibliography of his published work. A complete bibliography, prepared by Dr. Neiva and containing over 200 titles, is to be published in the forthcoming number from the Instituto Oswaldo Cruz.

Adolpho Lutz is survived by a sister, now in her ninety-second year, a son, Dr. Gualter Adolpho Lutz, Professor of Forensic Medicine at the University of Brazil, and a daughter, Bertha Lutz, Chief Naturalist of the National Museum at Rio de Janeiro. His wife died in 1922. To his daughter, who had been his companion and associate in the investigations of his later years, I am indebted for much of the material in this article.

Dr. Lutz was a modest and gracious person. His long life of service and accomplishment was crowned with the highest honors, the affection of a host of friends, and the esteem of scientists and medical men everywhere.—HORACE W. STUNKARD, *New York University*.

# AMERICAN SOCIETY OF PARASITOLOGISTS

## PRELIMINARY ANNOUNCEMENT OF THE SEVENTEENTH ANNUAL MEETING

MONDAY, TUESDAY AND WEDNESDAY, DECEMBER 29-31, 1941

DALLAS, TEXAS

In accordance with the decision of the Council, acting under authority voted at the sixteenth annual business meeting in Philadelphia, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in Dallas, Texas. Hotel Adolphus has been designated as official headquarters for the Society.

Sessions for the reading of contributed papers are scheduled on the first and third days. The main features of the program are arranged for the second day, Tuesday, December 30th. At the conclusion of the morning session, Dr. James E. Ackert will deliver the presidential address on the subject "Natural Resistance to Helminthic Infections." The annual Parasitologists' Luncheon will be held Tuesday noon and will be followed by the annual business meeting of the Society. In the afternoon, the demonstration program and tea will be held in the laboratories of Southern Methodist University.

The call for papers has already been mailed to members of the Society. In preparing abstracts, members are requested to consult the styling used in the December, 1940, Supplement of the Journal in order that editing may be kept at a minimum. Members are reminded that all abstracts for the program must be in the office of the Secretary not later than Friday, October 24, 1941.

O. R. McCoy. *Secretary*

# The Journal of Parasitology

Volume 27

December Supplement, 1941

## PROGRAM AND ABSTRACTS OF THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

DALLAS, TEXAS

DECEMBER 29, 30 and 31, 1941

### PROGRAM<sup>1</sup>

MONDAY MORNING SESSION, DECEMBER 29, 9:30 A.M.; ROOM G, FIRST  
METHODIST CHURCH, ROSS AVENUE AND HARWOOD STREET.

#### Read

1. Development of the Excretory System in Cercariae of Digenetic Trematodes. (10 min) (Lantern) KATHLEEN L. HUSSEY, Colorado College.

2. The Excretory System of *Paragonimus*. (8 min) (Lantern) ELON E. BYRD, University of Georgia.

3. Preliminary Report on *Echinochasmus* sp. (Trematoda), and a Comparison of the Excretory System of the Cercaria with Three Related Species. (10 min) (Lantern) (Also by demonstration) ELSIE W. TOWNSEND, Wayne University.

4. The Designation of Type Specimens in Describing New Species. (5 min) ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

5. Studies on Cercariae of the Common Mud-flat Snail, *Cerithidea californica*. I. The Pleurolophocercous Groups. (15 min) (Lantern) WANDA SANBORN HUNTER, University of California at Los Angeles.

6. The Early Developmental Stages of *Plagiorchis muris* (Trematoda: Plagiorchiidae) in Its First Intermediate Host. (15 min) (Lantern) W. W. CORT, Johns Hopkins University, AND LOUIS OLIVIER, U. S. Bureau of Animal Industry.

7. The Life History of *Apophallus brevis* Ransom, 1920. (15 min) (Lantern) MAX J. MILLER, Institute of Parasitology, Macdonald College, Quebec, Canada.

<sup>1</sup> An alphabetical author index will be found at the end of this program.

Extra copies of this Supplement, and portraits of parasitologists, will be on sale at the meeting.



8. The Life History of *Podocotyle atomon* (Rudolphi) (Trematoda: Opcoelidae). (10 min) (Lantern) (Also by demonstration) A. V. HUNNINEN AND R. M. CABLE, Oklahoma City University, Purdue University and the Marine Biological Laboratory.

9. Studies on the Life History of *Lecithaster confusus* Odhner (Trematoda: Hemiuridae). (10 min) (Lantern) A. V. HUNNINEN AND R. M. CABLE, Oklahoma City University, Purdue University and the Marine Biological Laboratory.

10. Studies on the Life History of *Siphodera vinalwardsii* (Linton) (Trematoda: Cryptogonimidae). (10 min) (Lantern) (Also by demonstration) R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University and the Marine Biological Laboratory.

11. Studies on the Life History of a Dicrocoeliid Trematode of the Genus *Lyperosomum*. (12 min) (Lantern) J. FRED DENTON, Georgia Southwestern College.

#### By Title

12. The Occurrence of *Dicrocoelium dendriticum* in the United States. EMMETT W. PRICE AND WILLIAM D. KINCHELOW, U. S. Bureau of Animal Industry.

13. *Phyllodistomum coatneyi* n. sp., a Trematode from the Urinary Bladder of *Ambystoma maculatum* (Shaw). F. G. MESERVE, Macalester College.

14. The Systematic Position of the Genus *Deropristis* Odhner with Respect to a Proposed Revision of the Trematode Families Acanthocolpidae and Allocreadiidae. R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University and the Marine Biological Laboratory.

15. Notes on Embryonating Eggs of *Zygocotyle lunata* and on Their Preparation for Cytological Study. GABRIEL C. GODMAN AND CHARLES H. WILLEY, New York University.

16. Parasites of the Green Turtle, *Chelonia mydas* (L.), with Special Reference to the Rediscovery of Trematodes Described by Looss from This Host Species. ROSS F. NIGRELLI, New York Aquarium.

17. Studies on Host-parasite Reactions. V. The Integumentary Type of Strigeid Cyst. GEORGE W. HUNTER, III, Wesleyan University, AND WANDA S. HUNTER, University of California at Los Angeles.

MONDAY AFTERNOON SESSION, DECEMBER 29, 2:00 P.M.; ROOM G, FIRST METHODIST CHURCH, ROSS AVENUE AND HARWOOD STREET.

#### Read

18. The Incidence of *Trichomonas foetus* in Wisconsin Cattle. (10 min) BANNER BILL MORGAN, University of Wisconsin.

19. The Effects of Sulfaguanidine on Experimental Bovine Coccidiosis. (15 min) (Lantern) (Also by demonstration) DONALD C. BOUGHTON, U. S. Regional Animal Disease Research Laboratory, Auburn, Ala.

20. Immunization against Coccidiosis by the Use of X-ray Attenuated Oöcysts. (15 min) (Lantern) S. H. WAXLER AND C. A. HERICK, University of Wisconsin.

21. Hatching Ascaris Eggs in Vitro. (15 min) (Lantern) GEORGE L. GRAHAM, Rockefeller Institute for Medical Research, Princeton, N. J.

22. Some Clinical Aspects of Experimental Esophagostomiasis in Cattle. (10 min) (Lantern) JOHN S. ANDREWS, U. S. Bureau of Animal Industry.

23. The Biology and Ecology of the Snail, *Stagnicola bulimoides techella* (Hald.), Intermediate Host of *Fasciola hepatica* Linn., in South Texas. (15 min) (Lantern) O. WILFORD OLSEN, U. S. Bureau of Animal Industry.

24. Losses of *Haemonchus* Larvae Early after Their Administration to Susceptible Sheep. (15 min) (Lantern) NORMAN R. STOLL, Rockefeller Institute for Medical Research, Princeton, N. J., AND J. H. TETLEY, Massey Agricultural College.

25. The Effect of Milk Diet on the Development of *Haemonchus contortus* in Calves. (15 min) (Lantern) DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Ala.

26. Studies on Bovine Gastro-intestinal Parasites: VII. The Effects of a Low Plane of Nutrition (Cottonseed Hulls) on Immunity to the Stomach Worm (*Haemonchus contortus*). (15 min) ROY L. MAYHEW, Louisiana State University.

27. Further Data on the Blood Picture in Stomach Worm (*Haemonchus contortus*) Infections. (15 min) ELAINE T. DELAUNE AND ROY L. MAYHEW, Louisiana State University.

28. Notes on the Musculature of the Male Genitalia of *Haemonchus contortus*. (15 min) (Lantern) WILLIAM LOGAN THRELKELD AND M. E. HENDERSON, Virginia Agricultural Experiment Station.

#### By Title

29. Sulfaguanidine Feeding as a Control Measure for Cecal Coccidiosis of Chickens. MARION M. FARR AND REX W. ALLEN, U. S. Bureau of Animal Industry.

30. Effect of a Single Dose of Phenothiazine upon Egg Production and Viability of the Eggs of Swine Lungworms. L. A. SPINDLER AND KENNETH C. KATES, U. S. Bureau of Animal Industry.

31. Limited Tests of Phenothiazine as an Anthelmintic in Goats.

PAUL D. HARWOOD AND JAMES E. GUTHRIE, Dr. Hess and Clark Laboratories, Ashland, O.

32. Skin and Precipitin Tests for the Diagnosis of *Trichina* Infections in Grain-fed and in Garbage-fed Hogs. L. A. SPINDLER AND JOHN L. AVERY, U. S. Bureau of Animal Industry.

33. Reactions in Garbage-fed and in Grain-fed Hogs to Intracutaneous Injections of Various Diluents. JOHN L. AVERY, U. S. Bureau of Animal Industry.

34. Precipitate Formation around *Trichina* Larvae in Sera from *Trichina*-infected, and *Trichina*-free Hogs. L. A. SPINDLER, JOHN L. AVERY AND HARRY E. ZIMMERMAN, JR., U. S. Bureau of Animal Industry.

35. Experimental Infection of Pigs with the Swine Thorn-headed Worm, *Macracanthorhynchus hirudinaceus*. KENNETH C. KATES, U. S. Bureau of Animal Industry.

36. The Duration of Infectivity of Nematode Parasites of Cattle on Florida Pasture with Observations on Resistance of Calves to Natural Reinfection with *Haemonchus contortus*. DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Ala., AND LEONARD E. SWANSON AND TERRENCE J. DRAKE, University of Florida.

TUESDAY MORNING SESSION, DECEMBER 30, 9:30 A.M.; ROOM G, FIRST METHODIST CHURCH, ROSS AVENUE AND HARWOOD STREET.

*Read*

37. The Occurrence of Mites in Pinnipeds, including a New Species from the California Sea-lion, *Zalophus californianus*. (10 min) (Lantern) (Also by demonstration) WILLIS H. DOETSCHMAN, University of Southern California and Biological Research Institute, Zoological Gardens of San Diego.

38. Observations on the Duration of an Infection of *Hymenolepis nana* in Man. (10 min) (Lantern) WILLIAM HUGH HEADLEE, Purdue University.

39. The Effect of Host Age on the Number of *Trichinella spiralis* Recovered from Rats during the Early Period of Infection. (10 min) L. O. NOLF AND HERMAN ZAIMAN, University of Iowa.

40. The Effects of Immune Serum upon the Larvae of *Trichinella spiralis* in Vitro. (15 min) (Lantern) EDWARD P. OFFUTT, JR., University of Rochester.

41. Attempted Passive Transfer to Rats and Mice of Immunity to *Trichinella spiralis*. (10 min) (Lantern) O. R. MCCOY AND FRANKLYN F. BOND, University of Rochester.

42. The Protective Action of Normal Sheep Serum against Infections of *Trypanosoma duttoni* in the Mouse. (5 min) (Lantern)

WILLIAM H. TALIAFERRO AND YELENA PAVLINOVA OLSEN, University of Chicago.

43. The Trypanocidal Action of Sheep Serum on *Trypanosoma duttoni*. (10 min) (Lantern) WILLIAM H. TALIAFERRO AND LUCY GRAVES TALIAFERRO, University of Chicago.

44. Quantitative Relationships in Immunity to Hookworm. (15 min) (Lantern) G. F. OTTO, Johns Hopkins University.

TUESDAY MORNING, DECEMBER 30, 11:00 A.M.; ROOM G, FIRST METHODIST CHURCH, ROSS AVENUE AND HARWOOD STREET.

*Presidential Address*

45. Natural Resistance to Helminthic Infections. JAMES E. ACKERT, Kansas State College.

TUESDAY NOON, DECEMBER 30.

12:30 P.M. PARASITOLOGISTS' LUNCHEON, HOTEL BAKER.

1:30 P.M. ANNUAL BUSINESS MEETING.

TUESDAY AFTERNOON SESSION, DECEMBER 30, 3:00 P.M.; HYER HALL, SOUTHERN METHODIST UNIVERSITY. (Tea will be served.)

*By Demonstration*

3. Preliminary Report on *Echinochasmus* sp. (Trematoda), and a Comparison of the Excretory System of the Cercaria with Three Related Species. (Also read) ELSIE W. TOWNSEND, Wayne University.

8. The Life History of *Podocotyle atomon* (Rudolphi) (Trematoda: Opecoelidae). (Also read) A. V. HUNNINEN AND R. M. CABLE, Oklahoma City University, Purdue University and the Marine Biological Laboratory.

10. Studies on the Life History of *Siphodera vinalledwardsii* (Linton) (Trematoda: Cryptogonimidae). (Also read) R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University and the Marine Biological Laboratory.

19. The Effects of Sulfaguanidine on Experimental Bovine Coccidiosis. (Also read) DONALD C. BOUGHTON, U. S. Regional Animal Disease Research Laboratory, Auburn, Ala.

37. The Occurrence of Mites in Pinnipeds, including a New Species from the California Sea-lion, *Zalophus californianus*. (Also read) WILLIS H. DOETSCHMAN, University of Southern California and Biological Research Institute, Zoological Gardens of San Diego.

46. A Case of Intestinal Myiasis. MARCUS W. LYON, South Bend Clinic, AND JOHN D. MIZELLE, University of Notre Dame.

47. *Hexamita* in the Blood of a Mouse. JUSTIN ANDREWS AND MADGE REYNOLDS, Georgia Department of Public Health.

48. Two Unusual Trematodes. H. W. MANTER, University of Nebraska.

49. Two Species of *Porrocaecum* (Nematoda) from the Robin. J. DAN WEBSTER, Rice Institute.

50. The Genus *Gnathostoma* in North America. ASA C. CHANDLER, Rice Institute.

51. Demonstration of a New Parasitic Nematode from a Water Scavenger Beetle. A. C. TODD, University of Nebraska.

53. Some Observations on *Histomonas* from Wild Pheasants and Domestic Fowls. (Also read) D. H. WENRICH, University of Pennsylvania.

62. Reservoir Hosts of Chagas' Disease in the State of Texas. (Also read) A. PACKCHANIAN, School of Medicine, University of Texas.

WEDNESDAY MORNING SESSION, DECEMBER 31, 9:30 A.M.; ROOM G,  
FIRST METHODIST CHURCH, ROSS AVENUE AND HARWOOD STREET.

*Read*

52. The Host-parasite Phylogenetic Complex: The Criteria and Their Application to the Opalinid-Anuran Complex. (15 min) JOHN LUTHER MOHR, University of California.

53. Some Observations on *Histomonas* from Wild Pheasants and Domestic Fowls. (15 min) (Lantern) (Also by demonstration) D. H. WENRICH, University of Pennsylvania.

54. The Pathogenicity for Kittens of *Endamoeba histolytica* from a Human Case of Acute Amoebic Dysentery. (15 min) (Lantern) CHARLES W. REES AND LUCY V. REARDON, National Institute of Health.

55. Pantothenic Acid and *Eimeria nieschulzi* Infection in the White Rat. (12 min) (Lantern) ELERY R. BECKER, Iowa State College.

56. Transmission of the Liver Coccidium, *Eimeria stiedae*, from the Domestic to the Cottontail Rabbit. (10 min) (Lantern) HARRY A. JANKIEWICZ, University of Southern California.

57. Life Cycles of Four Species of Intestinal Coccidia of the Domestic Rabbit. (15 min) (Lantern) ROBERT L. RUTHERFORD AND JOHN F. KESSEL, University of Southern California.

58. A Study of the Paroxysms Resulting from Induced Infections of *Plasmodium vivax*. (12 min) (Lantern) G. ROBERT COATNEY AND MARTIN D. YOUNG, U. S. Public Health Service.

59. Saurian Malaria. (10 min) (Lantern) CLAY G. HUFF, University of Chicago.

60. Blood-cell Counts in *Plasmodium durnae* Infections. (10 min) (Lantern) CARLTON M. HERMAN AND JEANNE YOUNG, Los Angeles Wildlife Disease Research Station.

61. The Anti-malarial Effect of Acranil. (15 min) (Lantern)



WENDELL D. GINGRICH AND ROLLIN S. FILLMORE, School of Medicine, University of Texas.

62. Reservoir Hosts of Chagas' Disease in the State of Texas. (15 min) (Motion Picture) (Also by demonstration) A. PACKCHANIAN, School of Medicine, University of Texas.

*By Title*

63. The Occurrence of *Heterodoxus longitarsus* Piaget (Mallophaga; Boopinae) on Dogs in Mississippi. JAMES W. WARD, Mississippi State College.

64. Mechanical Transmission of Shope Rabbit Fibroma by Certain Haematophagous Bugs. CORNELIUS B. PHILIP, Hamilton, Montana.

65. Oxidation-reduction Potentials in Relation to the Cultivation of *Endamoeba histolytica*. LEON JACOBS, National Institute of Health.

66. Indicanuria and the Vitamin A Absorption Test in Giardiasis. C. P. KATSAMPES, W. A. PHILLIPS, A. B. MCCOORD AND S. T. HARRIS, University of Rochester. (Introduced by O. R. McCoy.)

67. Incidence of Protozoan Infection amongst the Personnel of a Large General Hospital with Special Regard to Incidence according to Race and Occupation. MAURICE M. ROTHMAN AND MARION LASKEY, University of Pennsylvania.

68. Survival Time of Intravaginally Implanted *Trichomonas hominis*. ROBERT M. STABLER, University of Pennsylvania, AND L. G. FEO, Jefferson Medical College Hospital.

69. Notes on a Microsporidian Parasite, *Duboscqia* sp., of *Reticulitermes* of Maryland. RICHARD R. KUDO, University of Illinois.

WEDNESDAY AFTERNOON SESSION, DECEMBER 31, 2:00 P.M.; ROOM G, FIRST METHODIST CHURCH, ROSS AVENUE AND HARWOOD STREET.

*Read*

70. The Fate of Some Species of Schistosome Cercariae in Chick Embryos. (10 min) (Lantern) STERLING BRACKETT AND ALBERT J. BECKMANN, University of North Carolina and University of Michigan Biological Station.

71. Studies on Host-parasite Reactions. VI. An Hypothesis to Account for Pigmented Metacercarial Cysts in Fish. (15 min) (Lantern) GEORGE W. HUNTER, III, Wesleyan University.

72. Observations on the Geographical Distribution of Digenetic Trematodes of Marine Fishes. (10 min) (Lantern) H. W. MANTER, University of Nebraska.

73. The Frequency of Helminths in Their Naturally Infected Hosts.

(15 min) (Lantern) H. F. HSÜ AND S. Y. LI, Peiping Union Medical College, Peiping, China. (Introduced by the Secretary.)

74. Further Studies on the North American Hirudinea. (12 min) MARVIN C. MEYER, New Jersey College for Women of Rutgers University.

75. A New Species of Branchiobdellid from Kentucky. (8 min) CLARENCE J. GOODNIGHT, Brooklyn College.

76. Life History and Development of *Macracanthorhynchus ingens* and *Mediorhynchus grandis* (Acanthocephala). (10 min) (Lantern) DONALD V. MOORE, Rice Institute.

77. An Addition to the Life History of *Leidynema appendiculatum* (Leidy, 1850) Chitwood, 1932, a Nematode Parasitic in Cockroaches. (5 min) A. C. TODD, University of Nebraska.

78. Removal of Chicken Tapeworms by Host Starvation and Some Effects of Such Treatment on Tapeworm Metabolism. (15 min) (Lantern) W. M. REID, Monmouth College, AND J. E. ACKERT, Kansas State College.

79. Effect of Atabrine upon Experimental Cysticercosis of Mice. (12 min) (Lantern) JAMES T. CULBERTSON AND SYLVIA H. GREENFIELD, College of Physicians and Surgeons, Columbia University.

#### By Title

80. The Life Cycle of *Diocotophyma renale*, the Giant Kidney Worm of Mammals. ARTHUR E. WOODHEAD, University of Michigan.

81. The Possibility of Chemical Control of the Snail Intermediate Hosts of *Schistosoma mansoni* in Venezuela. GEORGE W. LUTTERMOSER, Instituto Nacional de Higiene, Caracas, Venezuela.

82. Effects of Duodenal Mucus of Dogs and Swine upon the Viability of *Ascaridia lineata* in Vitro. L. L. EISENBRANDT, University of Kansas City, AND J. E. ACKERT, Kansas State College.

83. The Rôle of Duodenal Mucus in Age Resistance. L. P. FRICK AND J. E. ACKERT, Kansas State College.

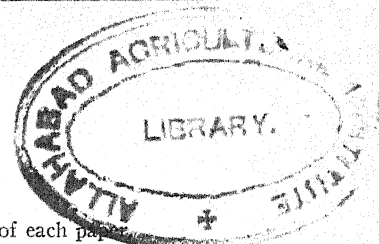
84. Intestinal Resistance to a Second Infection of *Trichinella spiralis*. ARTHUR J. LEVIN AND TITUS C. EVANS, University of Iowa.

85. Demonstration of Naturally Acquired, Passive Immunity to *Hymenolepis nana* var. *fraterna*. JOHN E. LARSH, JR., Johns Hopkins University.

86. Effects of Testosterone on Nematodes. LYELL J. THOMAS, University of Illinois.

87. Hairworms as Parasites of Fishes. ROSS F. NIGRELLI, New York Aquarium.

88. Cultivation Experiments with the Avian Malaria, *Plasmodium circumflexum*. FREDERICK COULSTON, Syracuse University.



## AUTHOR INDEX

Showing program number, which is also abstract number of each paper

<i>Author</i>	<i>Program &amp; Abstract Number</i>	<i>Author</i>	<i>Program &amp; Abstract Number</i>
Ackert, J. E. ....	45, 78, 82, 83	Levin, Arthur J. ....	84
Allen, Rex W. ....	29	Li, S. Y. ....	73
Andrews, John S. ....	22	Luttermoser, George W. ..	81
Andrews, Justin ....	47	Lyon, Marcus W. ....	46
Avery, John L. ....	32, 33, 34	Manter, H. W. ....	48, 72
Becker, Elery R. ....	55	Mayhew, Roy L. ....	26, 27
Beckmann, Albert J. ....	70	McCoord, A. B. ....	66
Bond, Franklyn F. ....	41	McCoy, O. R. ....	41
Boughton, Donald C. ....	19	McIntosh, Allen ....	4
Brackett, Sterling ....	70	Meserve, F. G. ....	13
Byrd, Elon E. ....	2	Meyer, Marvin C. ....	74
Cable, R. M. ....	8, 9, 10, 14	Miller, Max J. ....	7
Chandler, Asa C. ....	50	Mizelle, John D. ....	46
Coatney, G. Robert ....	58	Mohr, John Luther ....	52
Cort, W. W. ....	6	Moore, Donald V. ....	76
Coulston, Frederick ....	88	Morgan, Banner Bill ....	18
Culbertson, James T. ....	79	Nigrelli, Ross F. ....	16, 87
Delaune, Elaine T. ....	27	Nolf, L. O. ....	39
Denton, J. Fred ....	11	Offutt, Edward P., Jr. ....	40
Doetschman, Willis H. ....	37	Olivier, Louis ....	6
Drake, Terrence J. ....	36	Olsen, O. Wilford ....	23
Eisenbrandt, L. L. ....	82	Olsen, Yelena Pavlinova ..	42
Evans, Titus C. ....	84	Otto, G. F. ....	44
Farr, Marion M. ....	29	Packchanian, A. ....	62
Feo, L. G. ....	68	Philip, Cornelius B. ....	64
Fillmore, Rollin S. ....	61	Phillips, W. A. ....	66
Frick, L. P. ....	83	Porter, Dale A. ....	25, 36
Gingrich, Wendell D. ....	61	Price, Emmett W. ....	12
Godman, Gabriel C. ....	15	Reardon, Lucy V. ....	54
Goodnight, Clarence J. ....	75	Rees, Charles W. ....	54
Graham, George L. ....	21	Reid, W. M. ....	78
Greenfield, Sylvia H. ....	79	Reynolds, Madge ....	47
Guthrie, James E. ....	31	Rothman, Maurice M. ....	67
Harris, S. T. ....	66	Rutherford, Robert L. ....	57
Harwood, Paul D. ....	31	Spindler, L. A. ....	30, 32, 34
Headlee, William Hugh ...	38	Stabler, Robert M. ....	68
Henderson, M. E. ....	28	Stoll, Norman R. ....	24
Herman, Carlton M. ....	60	Swanson, Leonard E. ....	36
Herrick, C. A. ....	20	Taliaferro, Lucy Graves ...	43
Hsü, H. F. ....	73	Taliaferro, William H. ...	42, 43
Huff, Clay G. ....	59	Tetley, J. H. ....	24
Hunninen, A. V. ....	8, 9, 10, 14	Thomas, Lyell J. ....	86
Hunter, George W., III ...	17, 71	Threlkeld, William Logan. 28	
Hunter, Wanda S. ....	5, 17	Todd, A. C. ....	51, 77
Hussey, Kathleen L. ....	1	Townsend, Elsie W. ....	3
Jacobs, Leon ....	65	Ward, James W. ....	63
Jankiewicz, Harry A. ....	56	Waxler, S. H. ....	20
Kates, Kenneth C. ....	30, 35	Webster, J. Dan ....	49
Katsampes, C. P. ....	66	Wenrich, D. H. ....	53
Kessel, John F. ....	57	Willey, Charles H. ....	15
Kinchelow, William D. ...	12	Woodhead, Arthur E. ....	80
Kudo, Richard R. ....	69	Young, Jeanne ....	60
Larsh, John E., Jr. ....	85	Young, Martin D. ....	58
Laskey, Marion ....	67	Zaiman, Herman ....	39
		Zimmerman, Harry E., Jr. 34	

## ABSTRACTS

1. *Development of the Excretory System in Cercariae of Digenetic Trematodes.*

KATHLEEN L. HUSSEY, Colorado College.

The study of development of the excretory system of digenetic trematodes is continued with five species, including representatives of three families not previously studied. The development of the excretory system of *Proterometra macrostoma* (Azygiidae) is like that described for cercariae of Strigeata. Two primitive tubes form, extend to the tips of the furcae, fuse to form the bladder at the posterior end of the body and anterior end of the tail, then remain separate for the rest of the length of the tail. The mature cercaria has numerous caudal flame cells. In the gasterostome cercaria *Bucephalus elegans* (Bucephalidae) early development of the excretory system follows this same plan. After formation of the bladder each caudal tube makes contact with the surface of the tail fork near its proximal end forming one pore of the mature cercaria, and original caudal pores are lost. In the cercariaeum of *Zoogonus rubellus* (Zoogonidae) the excretory system develops as in stylet cercariae and similar to the cercariaeum of *Triganodistomum mutabile* as described by Wallace (1941, Tr. Am. Micr. Soc. 60: 322-324). Two primitive tubes extend to the posterior end of the body, fuse to form a bladder, and penetrate a mesodermal cell mass which forms the epithelial wall. Except for a much less distinct epithelial bladder wall fundament, the development of the excretory system of *Halipegus eccentricus* (Hemiuridae) is like that already described for *H. occidialis*. The development of the excretory system in *Himasthla quissetensis* (Echinostomidae) is like that of *Echinostoma revolutum* and *Petasiger nitidus*.

2. *The Excretory System of Paragonimus.* ELON E. BYRD, University of Georgia.

The excretory bladder terminates near the posterior end of the body where it communicates with the pore by an elongated, slightly muscular tube. The bladder is large and occupies a medial position in the body throughout its course, from about one-third the distance from the bifurcation of the ceca to the ventral sucker. The common collecting tubules contact the bladder between the levels of the ovary and testes and these tubules curve posteriorly, then laterally and finally anteriorly before giving rise to the anterior and posterior main tubules. Each of the anterior main collecting tubules gives rise to seven accessory tubules while each of the posterior main tubules gives rise to nine accessories. There is a decided tendency for the accessory tubules to take their origin in pairs. However, the third accessory from the anterior end of the anterior main and the third accessory from the posterior end of the posterior main tubules are single. Approximately half of the accessory tubules, one member of each pair, turns ventrally while the other member of the pair turns dorsally, and these supply the capillary tubules and flame cells for the dorsal and ventral aspects of the body. Each accessory tubule terminates by giving rise to three capillary tubules, each of which ends in a more or less stellate-shaped flame cell. Thus the formula for the excretory pattern is:  $2[(3+3+3+3+3+3+3) + (3+3+3+3+3+3+3+3+3)] = 96$  flame cells.

3. *Preliminary Report on Echinochasmus sp. (Trematoda), and a Comparison of the Excretory System of the Cercaria with Three Related Species.* ELSIE W. TOWNSEND, Wayne University.

The cercaria of *Echinochasmus* sp. was found in *Goniobasis livescens* collected from Douglas Lake, Michigan, and several localities along the Huron River near Ann Arbor, Michigan. This large-tailed echinostome cercaria is an active swimmer and acts as attractive bait to two species of dace and several species of minnows of the genus *Notropis*. In the fish host the metacercariae are found in ovoid thin-walled cysts embedded in the base of the gills. The collar spines begin

to appear in four or five days. Metacercariae are infective in 28 days, and when fed to domestic ducks develop to maturity in the small intestine. Maturity is reached in five to seven days. The small adult fluke has a single row of 22 collar spines interrupted dorsally by a space the width of the oral sucker. A study of the excretory system was made from the developmental stages of the cercaria beginning with the early embryo. As the tail develops the primary tubes are carried along and open laterally near the tip. The primary tubes fuse in the bladder region and in the tail. The bladder forms a body chamber and a tail reservoir. When the lateral excretory pores are established at the base of the tail, the caudal canals gradually become suppressed.

4. *The Designation of Type Specimens in Describing New Species.* ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

An examination of taxonomic papers published by members of the American Society of Parasitologists and others reveals a lack of uniformity in terminology when designating type specimens of new species. Frizzell (1933, *Am. Midland Naturalist* 14: 637-668), listing over 200 terms for type designations, called attention to this common fault of taxonomists. In some papers authors designate as *types* all specimens examined in the preparation of a species description; some designate only *cotypes*; an occasional author may designate *cotypes* and *paratypes* for a single species; and in proposing several new species in a single paper one author designated only *cotypes* for some species and *type* and *paratypes* for the remaining ones. Of common occurrence are papers having no designation of types. Every proposed new species or subspecies should be fixed by being anchored to a single *type* specimen. If the species is based on several specimens, having no single specimen designated as *type*, there is a chance that the lot of specimens may represent more than one species, thus creating confusion as to the status of the species originally proposed. Both confusion and lack of uniformity may be avoided by following the recommendations given in the second paragraph of Appendix "A" of the International Rules of Zoological Nomenclature, which is as follows: "It is recommended that in published descriptions of a new species or new subspecies, only one specimen be designated and labeled as *type*, the other specimens examined by the author at the same time being *paratypes*."

5. *Studies on Cercariae of the Common Mud-flat Snail, Cerithidea californica.* I. *The Pleurolophocercous Groups.* WANDA SANBORN HUNTER, University of California at Los Angeles.

*Cerithidea californica*, which is a characteristic inhabitant of the mud flats of southern California, acts as host for a large variety of cercariae. The pleuro- and parapleurolophocercous groups were found to include approximately 27 per cent of the total infection found in several large collections. The highest seasonal incidence for these groups comes in April and is coincident with the highest general infection. Although June is the low point for general infection, these groups comprised over 50 per cent of all of the types of cercariae found. It is believed that there are at least five different cercariae present in the author's collection from this host which belong to the Superfamily Opisthorchioidea. Methods of differentiating these species to facilitate later studies are being worked out. The excretory pattern cannot be used easily as a criterion in identifying them. Specific larval characters such as the number of penetration glands, the arrangement of the caudal finfolds, body proportions and especially the living habits must be relied upon.

6. *The Early Developmental Stages of Plagiorchis muris (Trematoda: Plagiorchiidae) in Its First Intermediate Host.* W. W. CORT, Johns Hopkins University, AND LOUIS OLIVIER, U. S. Bureau of Animal Industry.

Later stages of mother sporocysts of *P. muris* are oval, disc-shaped masses (0.5-1.7 mm), composed almost entirely of daughter sporocyst embryos (about 300



to 500 in each) attached to the outside of the snail's intestine. The matrix surrounding the embryos consists of irregular cells with no obvious organization. In earlier stages tubules composed of these cells can be made out enclosing linear series of the embryos. These same cells also form an outer covering for each daughter sporocyst, the "paletot" of earlier workers. The largest daughter sporocysts still found in the mother sporocyst masses are elongate and mobile, and contain cercarial embryos of different ages and a single discrete germ mass. When the mother sporocyst breaks up the daughters migrate along the ducts and vessels to all parts of the digestive gland of the snail. They then become attached, thicken, and appear crowded with embryos. Thickened areas of the true sporocyst wall are formed at their ends and from them protrusions push out. Further growth of these protrusions and the pushing into them of cercarial embryos produce a variety of irregularly shaped sporocysts, each loosely enclosed in the "paletot." Later, with increasing size and rapid growth of the cercarial embryos the shapes of the sporocysts become less irregular. Growth continues after the escape of the first mature cercariae, and later the outer layer becomes pigmented. The germ masses persist throughout the whole life of the daughter sporocysts, and are found still apparently producing new cercarial embryos in the oldest sporocysts from old snails.

7. *The Life History of Apophallus brevis* Ransom, 1920. MAX J. MILLER, Institute of Parasitology, Macdonald College, Quebec, Canada.

A re-study of the morphology of *Apophallus imperator* Lyster, 1940, and *A. brevis* Ransom, 1920, shows the two species to have no constant differences of specific importance. The appearance of the gonotyle, which Lyster used as a character to separate these species, cannot be accepted as sufficiently significant or constant to be of specific importance. *A. imperator*, therefore, falls as a synonym of *A. brevis*.

Studies on the life history of *Apophallus brevis* in Quebec, Canada, have incriminated the small operculate snail, *Amnicola limosa*, as the first intermediate host. The second intermediate host has been found in nature to be the speckled trout (*Salvelinus fontinalis*). The metacercariae encyst in the skin, forming a black pigmented cyst. These cysts have never been found in the muscle tissue. The metacercariae become infective to the definitive host within 3 to 4 weeks. Speckled and brown trout have been infected experimentally. Rainbow trout, however, are refractory to infection. The metacercariae will develop to an adult stage in pigeons and kittens, under experimental conditions, in from 2 to 7 days. Ransom found adult *A. brevis* in the California gull. However, in Quebec the normal host in nature has been shown to be the loon (*Gavia immer*). Young loons were successfully infected experimentally, and have been found infected in nature. There is some evidence that old loons may be resistant to infection.

8. *The Life History of Podocotyle atomon* (Rudolphi) (Trematoda: Opecoelidae). A. V. HUNNINEN AND R. M. CABLE, Oklahoma City University, Purdue University, and the Marine Biological Laboratory.

Experimental studies have demonstrated that the cercaria of *Podocotyle atomon* is a cotylomicrocercous larva and not a trichocercous form as suggested by Palombi (1938). It develops in *Littorina rudis* and is very similar to the cercaria of *Opecoeloides manteri*, both larvae having double-pointed stylets, three pairs of cephalic glands, and an excretory formula of  $2[(2+2) + (2+2)]$ . Chief differences are in respect to sucker size and the tail; in *P. atomon*, the ventral sucker of the cercaria is larger than the oral sucker and the tail lacks a protrusible glandular core as in the cercaria of *O. manteri*. The marine amphipods, *Gammarus* sp., *Carinogammarus mucronatus*, and *Amphithoë longimana*, serve as intermediate hosts. Of these, the last two are rarely infected in nature but serve readily as experimental hosts. At an age of four weeks, the cyst is 0.3 mm in diameter and the digestive and reproductive systems of the metacercaria are well formed. Old cysts may measure 0.6 mm in diameter and the enclosed worms approach adults in size and

development, becoming sexually mature and producing eggs. The excretory formula is unchanged during post-cercarial development. A number of marine fishes serve as natural definitive hosts. Similarities in fundamental respects demonstrate that *Podocotyle* and *Opecoeloides* are closely related, and that certain differences between their adult stages such as the presence or absence of a cirrus sac, accessory suckers, and junction of the ceca with the excretory vesicle are minor characters probably of no greater than generic value in the Opecoelidae.

9. *Studies on the Life History of Lecithaster confusus* Odhner (Trematoda: Hemiuridae). A. V. HUNNINEN AND R. M. CABLE, Oklahoma City University, Purdue University, and the Marine Biological Laboratory.

The cercaria of *Lecithaster confusus* is a minute, simplified cystophorous larva developing in the marine snail, *Odostomia* sp. The cyst is discoidal, measuring 0.05 mm in diameter. It contains the cercarial body and inverted delivery tube. Light cover glass pressure causes sudden eversion of the tube and passage of the body through it so rapidly as to be followed only with difficulty. The cercaria is unable to swim, since its only appendage is a simple, delicate, non-motile filament. After escape from the cyst, the cercarial body measures 0.085 mm long. Its structure is poorly defined, even the suckers being indistinct. The excretory vesicle with paired main tubules extending half the length of the body may be observed in favorable specimens. Development of the cercaria from the germ ball has been traced.

Cercariae are eaten by copepods. Twenty per cent of the *Acartia* sp. collected in the vicinity of infected snails were naturally infected. Metacercariae develop rapidly and occur free in the body cavity of the copepod. In 6-day metacercariae, the two main excretory tubules reach the pharyngeal level where their expanded terminations are connected by a cross-commissure which, unlike that of most hemiurids, disappears in the adult stage while another develops near the posterior end of the body. Sticklebacks were used as experimental definitive hosts although many marine fishes may harbor *L. confusus*. The excretory pattern of the adult has been determined.

10. *Studies on the Life History of Siphodera vinalwardsii* (Linton) (Trematoda: Cryptogonimidae). R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University and the Marine Biological Laboratory.

The cercaria of *Siphodera vinalwardsii* is a pleurolophocercous larva developing in rediae in the marine snail, *Bittium alternatum*, from the Woods Hole region. It has 14 cephalic glands with two instead of the usual four bundles of ducts. The tail is inserted ventrally and is coiled when the larva is at rest. The excretory formula is  $2[(2+2) + (2+2)]$ . The cercaria was found to penetrate and encyst in flounders more readily than in other fishes tried. Metacercariae must be over 9 days of age to establish an infection when fed to toadfish, the natural definitive host. The present study supports the view that *Siphodera* and related trematodes have close affinities with the Heterophyidae and should be included in the superfamily Opisthorchioidea. Because of similarities between the cercaria of *S. vinalwardsii* and *Cercaria coronanda* Rothschild, which has been referred to the Acanthostomidae, separation of the families Acanthostomidae and Cryptogonimidae may be unwarranted.

11. *Studies on the Life History of a Dicrocoeliid Trematode of the Genus Lyperosomum*. J. FRED DENTON, Georgia Southwestern College.

An undescribed trematode belonging to the genus *Lyperosomum* Looss, 1899, was found to be a common parasite in the livers of meadowlarks, *Sturnella magna argutula*, and bronzed grackles, *Quiscalus quiscula aeneus*, in the vicinity of Houston, Texas. Incubated eggs from the uteri of mature worms of this species were fed to parasite-free specimens of 7 common species of land snails. The eggs hatched in all 7 snails but development to maturity proceeded only in *Polygyra texasiana* (Moricand) and *Praticollega berlandieriana* (Moricand). Hatching of the eggs

occurs in the upper small intestine from which the miracidia pass to the digestive gland to transform into mother sporocysts. Each mother sporocyst produces a single generation of 50 to 70 daughter sporocysts which it liberates into the haemocoelic space of the snail by rupturing. The daughter sporocysts produce long-tailed, stylet cercariae which migrate out of the daughter sporocysts through the cervical birth canal and actively work their way into the mantle cavity of the host. Here the cercariae collect in masses of 150 to 300, the masses being held together by a slimy material secreted from their large cystogenous glands. These cercarial masses are expelled from the respiratory chamber and are deposited on plants and other objects as the snails crawl along. In the laboratory cultures cercarial masses were first expelled by the snails on the 106th day after infection. Larvae of the chrysomelid beetle, *Gastroidia cyanea*, exposed to freshly expelled cercariae on the leaves of dock (*Rumex* sp.), contained 1 to 6 non-encysted worms at autopsy. No attempts were made to infect the definitive hosts.

12. *The Occurrence of Dicrocoelium dendriticum in the United States.* EMMETT W. PRICE AND WILLIAM D. KINCHELOW, U. S. Bureau of Animal Industry.

Up to the present time there has been no authentic report of the occurrence of the lancet fluke, *Dicrocoelium dendriticum*, in the United States, although Leidy (1856) noted that this fluke is "Stated to be frequent in the sheep in several of the Western States," and several treatises on parasites have listed the United States as one of the countries in which this species is found. During the past year eight cases of parasitism with the lancet fluke have been encountered in cattle slaughtered at Abattoir No. 1016, Newark, N. J. Four of these were found by the junior author and the remaining cases by Dr. S. R. Roberts. The identity of specimens from all of the cases was checked by the senior author. Through the efforts of Dr. L. V. Hardy, inspector in charge of this establishment, the origin of the parasitized cattle was traced to the state of New York, three of the animals being traced to farms in Cayuga and Portland counties. From information secured from the owners there is no doubt that these were native cases and that the parasite has definitely become established in the state mentioned.

13. *Phyllodistomum coatneyi* n. sp., a Trematode from the Urinary Bladder of *Ambystoma maculatum* (Shaw). F. G. MESERVE, Macalester College.

Two out of 19 specimens of *Ambystoma maculatum* were infected with 10 parasites. They were collected at Bass Lake, Wisconsin. *P. coatneyi* is most like *P. americanum*. The vitellaria of *P. americanum* are approximately the same size as the ovary whereas in *P. coatneyi* the vitellaria are only about one-half the size of the ovary. Length 3.3–7.0 mm; width 0.7–1.7 mm; oral sucker 0.301–0.473 mm; acetabulum 0.455–0.636 mm; testes irregularly and deeply lobed; eggs non-operculate 0.021–0.029 mm in length by 0.015–0.020 mm in width. The nearest relative, *P. americanum*, has eggs which are 0.052 by 0.050 mm. *P. coatneyi* is named in honor of Dr. G. R. Coatney of the U. S. Public Health Service.

14. *The Systematic Position of the Genus Deropristis Odhner with Respect to a Proposed Revision of the Trematode Families Acanthocolpidae and Allocreadiidae.* R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University, and the Marine Biological Laboratory.

Studies on the life history of *Deropristis inflata* demonstrate that the family Acanthocolpidae is not a natural group of closely related trematodes. A redefinition of the family in the light of recent work would exclude the genus *Deropristis* and *Dihemistephanus sturionis* while the genera *Lepidauchen*, *Pleorchis*, and *Pseudolepidapedon* would be included for the first time.

The nearest known relatives of *Deropristis* are undoubtedly members of the Anallocreadiinae. The subfamilies Anallocreadiinae, Lepocreadiinae and a new one to include *Deropristis* are regarded as a family Lepocreadiidae distinct from the Allocreadiidae, which should be restricted to forms having ophthalmoxiphidiocer-

cariae developing in bivalves. The Acanthocolpidae and Allocreadiidae, as thus restricted, are regarded as separate but fairly closely related groups, possibly more so than any two subfamilies hitherto allocated to the family Allocreadiidae (sensu lato).

There seems good reason to support Hopkin's (1941) allocation of trematodes having cotylomicrocerous cercariae to a separate family, Opecoelidae, and Manter's (1940) suggestion that the Gyliarchenidae is more closely related to the Lepocreadiinae than to the amphistomes. Superfamily relationships of the Allocreadiidae, Acanthocolpidae, Opecoelidae, Lepocreadiidae, and Gyliarchenidae are not apparent from the fragmentary knowledge of life histories and embryology, particularly of marine forms.

15. *Notes on Embryonating Eggs of Zygocotyle lunata and on Their Preparation for Cytological Study.* GABRIEL C. GODMAN AND CHARLES H. WILLEY, New York University.

Difficulty encountered from mold and bacteria on shells of embryonating eggs of *Zygocotyle lunata* is effectively overcome by keeping living Ostracoda with the eggs. After several washings, 30 to 50 eggs are placed in a covered Petri dish with from 10 to 15 ostracods which may be obtained from aquaria or cultured easily in water containing decaying lettuce. They hold and rotate the eggs with their appendages, effectively scraping off the fungi and leaving them clean and undamaged. The Ostracoda must be replaced and the water changed about every 5 to 7 days, since most of the crustacea die within that period. Ova of *Z. lunata* prepared for sectioning in early stages of development collapse immediately in most common fixatives (Bouins, Helly's, Zenker's, Gibson's). In Flemming's fluid they suffer no change of form, penetration has occurred within 10 minutes and fixation is complete within 30 minutes. Since collapse or rupture occurs in the usual series of alcohols, successful dehydration was accomplished only by the capillary-wick technique of McClung, by which a very gradual instillation of alcohol occurs. They collapse and rupture in pure dioxan, buckle slowly in 50 per cent dioxan, and its gradual addition offers no advantages over alcohol. Clearing must be accomplished slowly with xylol or chloroform by the capillary-wick technique. Gradual infiltration with the rubber-paraffin mixture of Hance is of value in reducing to a minimum the shattering of the shell that occurs in pure paraffin.

16. *Parasites of the Green Turtle, Chelonia mydas (L.), with Special Reference to the Rediscovery of Trematodes Described by Looss from this Host Species.* ROSS F. NIGRELLI, New York Aquarium.

Looss (1899, 1902) reported some 24 species of trematodes from *Chelonia mydas*. The following were recently recovered from the viscera of 50 turtles: (Pronocephalidae) *Cricocephalus albus* (Kuhl and Hasselt, 1822) and *C. megastomus* Looss, 1902 (stomach); *Pronocephalus obliquus* Looss, 1901 and *Glyphicephalus lobatus* Looss, 1901 (intestine); *Pyelosomum cochlear* Looss, 1899 (urinary bladder). A new species of *Charaxicephalus* Looss, 1901 was found in the stomach. No forms of *Pleurogonius* Looss, 1901 were present. *Deuterobaris proteus* (Brandes, 1891), *Octangium sagitta* (Looss, 1899), *Angiodictyum parallelum* (Looss, 1901) and *Polyangium linguatula* (Looss, 1899), all Angiodictyidae, were found in the large intestine. *Microscaphidium* Looss, 1900 is not represented. A specimen of *Schizamphistomoides spinulosum* (Looss, 1901) Stunkard, 1925 (Paramphistomidae) was recovered from the large intestine. Of the intestinal distomes reported by Looss, *Enodiotrema megachondrus* (Looss, 1899) (Plagiorchidae) was the only one found. Other distomes present in our collection are as follows: (Rhytidodidae) *Rhytidodoides intestinalis* Price, 1939, *R. similis* Price, 1939, and a new species of *Rhytidodoides* Price, all from gall bladders. The family Spirorchidae Stunkard, 1921, is represented in this collection by *Learedius leardi* Price, 1934, and *L. similis* Price, 1934, from the heart and visceral vessels. Large numbers of leeches, *Ozobranchus branchiatus* (Menzies, 1791), were found associated with fibro-epithelial tumors on eyelids. Heavy infections of *L. leardi* and *R. similis* occurred in 65 per

cent of the organs examined. In four cases, a papillomatous disease of the gall bladder (Smith, Coates and Nigrelli, 1941) was found associated with heavy infections of *R. similis*.

17. *Studies on Host-parasite Reactions. V. The Integumentary Type of Strigeid Cyst.* GEORGE W. HUNTER, III, Wesleyan University, AND WANDA SANBORN HUNTER, University of California at Los Angeles.

The integumentary type of cyst characteristic of *Crassiphiala bulboglossa* and *Necascus rhinichthysi* is described from the yellow perch, *Perca flavescens*, and the black-nosed dace, *Atratulus atronotus*, respectively. In both cases special connective tissue stains were used. An inner hyaline cyst is elaborated by the parasite while the host responds by producing a connective tissue cyst containing melanin and melanin-bearing cells resembling melanophores. These outer host cysts produced by the fish are, in general, less dense than those noted in the case of parasites penetrating to the deeper layers of the host, as *Uculifer ambloplitis*.

18. *The Incidence of Trichomonas foetus in Wisconsin Cattle.* BANNER BILL MORGAN, University of Wisconsin.

Examinations were made of the uteri from 560 Wisconsin dairy cows obtained from a local packing plant. Of the 560 uteri examined 416 were pregnant, 110 normal non-pregnant, 31 non-pregnant with pyometra, and 3 pregnant with pyometra. From the total number of uteri examined 8 or 1.4 per cent were positive for trichomonads. Of the 8 cases, the amniotic fluid of an apparently healthy seven-months foetus (approximate age) was teeming with *T. foetus*. From the 31 non-pregnant uteri with pyometra 5 were positive and from the 3 pregnant uteri with pyometra containing macerated foeti 2 were positive. The incidence of *T. foetus* from the 33 uteri with pyometra was 21 per cent.

Amniotic fluid, occasionally allantoic fluid, swabs from the mouth of each foetus, and exudate from uteri with pyometra were inoculated into culture media. The material was incubated at 30° C for 18 hours and examined for trichomonads. After the first examination the cultures were incubated at 37° C for 18 hours and examined again. The culture material was the all-autoclaved medium of Schneider (unpublished thesis). Occasionally egg slants layered with saline, Ringer's, or saline and serum were used.

Four cases of trichomoniasis have been positively diagnosed out of 28 herds representing a total number of approximately 500 dairy cows for an incidence of 0.8 per cent. This is a preliminary report, but the above figures indicate that trichomoniasis, at least in Wisconsin, is an important problem in the dairy cattle industry.

19. *The Effects of Sulfaguanidine on Experimental Bovine Coccidiosis.* DONALD C. BOUGHTON, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

In one experiment 9 calves were inoculated simultaneously with approximately 50 million sporulated oöcysts of mixed types from one large suspension. Four of the 9 received no drug. All of these developed clinical coccidiosis, *Eimeria bovis* being the dominant species. One was sacrificed when moribund 15 days after inoculation. The remaining three died on the 20th, 21st, and 27th days, respectively. Five calves were given at least 21 consecutive daily doses of sulfaguanidine at the rate of 0.1 gm per 2.2 pounds of body weight beginning 2 days after inoculation. The 5 treated calves survived, did not develop clinical coccidiosis, and discharged very few *E. bovis* oöcysts during the month following inoculation. In a second experiment 12 calves were given a similar inoculation of infective oöcysts from a second suspension. Three calves received no drug. Sulfaguanidine was given to the remaining 9 calves at the rate of 5.0 gms per day for 8 consecutive days as follows; three calves, at the time of inoculation; 3 calves, mid-way in the 3-week incubation period; and 3 calves, toward the end of the incubation period. All calves receiving the early and mid-way treatment and 2 of the 3 untreated calves developed



fatal coccidial infections, *E. bovis* being the dominant species. The surviving untreated calf developed *E. bovis* infection. The 3 calves treated late in the incubation period survived; none discharged large numbers of *E. bovis* oöcysts during the month following inoculation.

20. *Immunization against Coccidiosis by the Use of X-ray Attenuated Oöcysts.* S. H. WAXLER AND C. A. HERRICK, University of Wisconsin.

Chickens that are infected with x-ray attenuated oöcysts acquire a certain amount of resistance to a second heavy infection. This resistance, when measured by the hemoglobin level after infection, is almost as great as that afforded the survivors that had been infected originally with untreated oöcysts. The attenuated oöcysts not only gave protection against subsequent reinfection but were safe at the initial infection. It was, however, necessary to standardize the oöcyst dose in roentgen units for the different age groups of chickens. The younger birds required oöcysts attenuated for a slightly longer time than did the older ones. By varying the amount of radiation, it is possible to control fairly accurately the severity of the disease. The longer the oöcysts were subjected to the rays, the less severe was the disease. The use of x-rays as the attenuating agent appears to be quite satisfactory, in that results, as indicated by the hemoglobin change, can be duplicated by using the same quantity of radiation measured in roentgen units.

21. *Hatching Ascaris Eggs in Vitro.* GEORGE L. GRAHAM, Rockefeller Institute for Medical Research, Princeton, N. J.

It was shown by Graham (1937) that rats were inferior to guinea pigs for demonstrating lung hemorrhage due to migration of swine ascaris larvae following feeding of infective eggs. Experiments conducted to ascertain conditions under which hatching occurred in vivo, established that eggs introduced directly into the intestine of guinea pigs were effective in producing heavy lung damage, but not in rats. This demonstrated that hatching occurred normally in the guinea pig intestine at least, without the necessity of stomach passage.

In hatching experiments conducted in vitro, infective eggs were incubated at 38° C. Tubes containing increasing quantities of guinea pig bile showed a correlated increase in hatching when a small piece of guinea pig pancreas was present. Tubes containing bile alone, or pancreas alone, showed no evidence of hatching. The increased hatching with increasing amounts of bile in the presence of pancreatic enzymes is, interestingly enough, correlated with the absence of a gall bladder in the rat and its presence in the guinea pig.

22. *Some Clinical Aspects of Experimental Esophagostomiasis in Cattle.* JOHN S. ANDREWS, U. S. Bureau of Animal Industry.

At the Puerto Rico Agricultural Experiment Station it was found that administration of 20,000 to 260,500 infective larvae of *Oesophagostomum radiatum* by mouth to healthy calves weighing from 70 to 280 pounds produced transient rises in body temperature to 104° F or above, at approximately 4 and 10 days after infection, which appeared to be correlated with the presence of the newly arrived third stage larvae in the intestinal wall, and with the exit of the fourth stage larvae from the mucosa, respectively. Severe diarrhea, accompanied by anorexia and tenesmus, began 4 to 23 days after infection and continued for periods up to 186 days. A transient polymorphonuclear leucocytosis associated with the repair of intestinal tissue damaged or destroyed by the larvae occurred 2 to 4 weeks after completion of infection. A reduction of 50 per cent in the number of erythrocytes developed about 5 weeks after infection, the red blood cell count returning to normal with the disappearance of clinical symptoms. Absence of blood in the feces indicated that the anemia may have been due to the action of some toxin on the hematopoietic system. Difficulty was experienced in the early diagnosis of the disease because of the absence of worm eggs from the feces for a period of from 3 to 5 weeks after the onset of the diarrhea. The number of adult *O. radiatum* recovered post mortem did

not appear to bear any relation to the severity of the disease. Death occurred in one calf following infection with 82,000 larvae.

23. *The Biology and Ecology of the Snail, Stagnicola bulimoides techella* (Hald.), *Intermediate Host of Fasciola hepatica* Linn., in *South Texas*. O. WILFORD OLSEN, U. S. Bureau of Animal Industry.

The snail *Stagnicola bulimoides techella* (Hald.) is the most important intermediate host of the liver fluke in the coastal section of Texas. The composition of the snail population varies according to the season and its weather. Generally speaking, however, juvenile snails predominate in the fall, juveniles and adults being about equal during the winter and the adults exceeding in number during the spring. In one habitat, 92 per cent of the snails found dead on the surface after the pool had dried were adults while 98.6 per cent of the surviving population which reappeared with the advent of standing water after 169 days of drought were small juveniles. In habitats under conditions of more evenly distributed rainfall, adult snails aestivate commonly, even carrying infections of *Fasciola hepatica* through the period of dormancy. During rainy weather when pools contain water throughout the summer, reproduction is continuous. Snails occur in greatest numbers during the winter. Associated with a high reproductive potential is a short life cycle which may be completed in 21 days under optimum conditions. During a total of 334 egg-laying days extending through July, August and September, four laboratory snails deposited 17,432 ova. The mean number of ova per snail per day was 52.9, the daily mean number of egg masses 2.8 with a mean number of 18.5 ova per mass. Natural infection of snails with *Fasciola hepatica* taken from pastures during the current year when rainfall has been high and at frequent intervals, where cattle grazed constantly, was 0.51 per cent.

24. *Losses of Haemonchus Larvae Early after Their Administration to Susceptible Sheep*. NORMAN R. STOLL, Rockefeller Institute for Medical Research, AND J. H. TETLEY, Massey Agricultural College.

*Haemonchus contortus* in susceptible sheep represents a worm-host system characterized by a high degree of infectivity of the nematode and its marked pathogenicity for the host. Effective parasitization is favored because infective larvae, being swallowed, are brought in due course to the abomasum. Worms which pass beyond this 4th stomach are irretrievably lost (although such larvae, if still alive and promptly rescued from the fecal pellets, can be shown normally infective).

The present tests employed huge single doses of larvae. None was recovered the first six hours, but fecal collections the next two hours were already positive for larvae in some animals. Once demonstrated, larvae were found continually discharged during the first three days, the longest period studied. In one instance an animal sacrificed 72 hours after oral infection of 941,000 larvae showed respectively 18, 7, and 1 per cent of these in the three 24-hour periods preceding post mortem demonstration of 261,000 (28 per cent) established in the abomasum. The limiting factor did not appear to be their number in the abomasum. In two cases where over 319,000 were present there at the end of one day, about 1 per cent of inoculated larvae were demonstrable in feces before death; original dose was given orally in one case, injected directly into the rumen through the body wall in the other. While infection losses are not unlooked for, with *Haemonchus* they represent organisms which, after successfully reaching the abomasum, fail to remain in the one parasitic habitat where they can survive. Lost larvae are characteristically exsheathed.

25. *The Effect of Milk Diet on the Development of Haemonchus contortus in Calves*. DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

Three pairs of calves, kept under parasite-free conditions since birth, were used in these experiments. One calf of each pair was maintained on a control diet of

cow's milk, alfalfa hay and grain. The other calf of each pair was fed nothing but milk from the time of birth until the termination of the experiments, approximately a month after feeding infective larvae of *H. contortus*. The calves of the first pair were fed 98,000 larvae each; those of the second, 26,000; and those of the third, 10,500 when 4, 3, and 2 months old, respectively. Although the calves on the milk diet failed to develop properly in comparison with their controls they did not become anemic or more susceptible to parasitism as was anticipated from the results of previous work with milk diet and parasitism (Porter, 1935; Foster, 1936). At necropsy the calves in each pair that were fed only milk harbored 282, 346, and 0 worms, whereas their respective controls on a normal diet harbored 2,888, 8,202 and 887 worms. The worms from the milk-fed calves were also smaller than those from the calves on the regular diet. Failure of these blood-feeding parasites to develop in the milk-fed calves is possibly due to unfavorable environmental factors in the abomasum.

26. *Studies on Bovine Gastro-intestinal Parasites: VII. The Effects of a Low Plane of Nutrition (Cottonseed Hulls) on Immunity to the Stomach Worm (Haemonchus contortus)*. ROY L. MAYHEW, Louisiana State University.

The immunity to the stomach worm, *Haemonchus contortus*, has not been completely broken down and but little reduced by the feeding of cottonseed hulls to three calves. Each animal was demonstrated to be resistant by reinoculation, repeated egg counts, and post mortem examination.

27. *Further Data on the Blood Picture in Stomach Worm (Haemonchus contortus) Infections*. ELAINE T. DELAUNE AND ROY L. MAYHEW, Louisiana State University.

In addition to the description of the blood picture of three calves recorded previously (1940, J. Parasitol. 26 Suppl: 17) data on three more animals are here presented. The erythrocytes decreased approximately 2 to  $3\frac{1}{2}$  million below the 9,500,000-10,300,000 normals between the 5th and 8th days after inoculation. A further decrease of about 2 million occurs within 23-28 days after inoculation. The hemoglobin, likewise, decreases in a similar proportion on approximately the same days. The total leucocyte counts increase from the pre-inoculation normal (9,600-11,000) to 15,800 to 27,900 five to seven days after inoculation. A rapid decrease follows within the next 25 days to minimum counts of 1,700 to 7,000 in different animals. A much greater range of variation persisted during the entire period of observation in the hemoglobin determinations, total erythrocytes and leucocyte counts in all animals after inoculation than during the pre-inoculation period. There was an increase in the monocyte and a decrease in the lymphocyte percentages. But very slightly, 1 to 2 per cent, higher eosinophile percentages were obtained after inoculation than before.

28. *Notes on the Musculature of the Male Genitalia of Haemonchus contortus*. W. L. THRELKELD AND M. E. HENDERSON, Virginia Agricultural Experiment Station.

A study of longitudinal and cross sections through the posterior region of the male nematode, *H. contortus*, shows that the gubernaculum is supported and slightly activated by longitudinal and transverse muscles and that the location of these muscles implies that slight movement is possible in three directions. Other muscles are described and their functions are explained on the basis of their origins, insertions, and locations: (a) Muscles 1 and 3, each named *retractor spicularis lateralis*, function to retract the spicule. (b) Muscle 2, *retractor spicularis centralis*, functions to retract the spicule. (c) Muscle 4, *protractor spicularis*, functions to extend the spicule and assist in contraction of the bursa. (d) Muscle 5, *dilator cloacae*, functions to dilate the cloaca. (e) Muscle 6, *bursa expansa*, functions to expand the bursa.

Spermatazoa contained in gelatinous capsules are found in the grooves formed

by the longitudinal spicular ridges. Two pairs of glandular-like bodies are shown proximal to the intestine.

29. *Sulfaguanidine Feeding as a Control Measure for Cecal Coccidiosis of Chickens.* MARION M. FARR AND REX W. ALLEN, U. S. Bureau of Animal Industry.

Tests involving 100 chicks used as principals, and 100 inoculated and 25 uninoculated control chicks, were conducted to determine the value of sulfaguanidine as a prophylactic for *Eimeria tenella* infections. The birds were 12 to 16-day old Rhode Island Reds. One-half of the principals received a mash containing 1 per cent sulfaguanidine, and the other half received a mash containing 2 per cent of the drug for a period beginning 4 days before and ending 9 days after inoculation. Neither group exhibited symptoms of coccidiosis. A few coccidial stages were found in the group receiving the 1 per cent mash, but none was found in the group receiving the 2 per cent mash. All inoculated controls exhibited typical symptoms of cecal coccidiosis and 33 per cent died. The mean gains in weight of the various groups were as follows: One per cent sulfaguanidine group, 99.8 gm; 2 per cent group, 95.6 gm; inoculated controls, 76.8 gm; uninoculated controls, 118.2 gm. The results indicate the efficacy of the drug as a preventive.

In order to test the possible curative value of sulfaguanidine 25 chicks were given 3 per cent medicated mash and 25 were given 5 per cent medicated mash at the first sign of blood in the droppings. This treatment was continued for 6 days. Although the oöcyst production was sharply reduced by the treatments named, there was no evidence that the drug had a significant curative effect.

30. *Effect of a Single Dose of Phenothiazine upon Egg Production and Viability of the Eggs of Swine Lungworms.* L. A. SPINDLER AND KENNETH C. KATES, U. S. Bureau of Animal Industry.

A pig passing approximately 2,247,000 lungworm eggs per day (experimentally infected) was given 20 gms of phenothiazine mixed with feed. Each day thereafter, counts were made of the number of lungworm eggs in the feces. Earthworm-feeding tests of the viability of the eggs in representative 50-gram samples of feces were made prior to administration of the drug and each day thereafter. Prior to administration of phenothiazine the average infection acquired by individual earthworms varied from 400 to 500 lungworm larvae. Three days after treatment the number of lungworm eggs passed was approximately 1 million but in earthworm-feeding tests these annelids acquired an average infection of less than 1 larva each. Seven days after treatment the feces contained approximately 500,000 lungworm eggs and earthworms acquired average infections of more than 200 larvae each. By the eleventh day after treatment the number of lungworm eggs in the feces and the infections acquired by earthworms in the feeding tests had returned to near pre-treatment levels. Feces collected 3 days after administration of phenothiazine were exposed to prevailing weather conditions for 1 month on soil outdoors. In subsequent feeding tests each earthworm acquired an average infection of slightly less than 200 larvae. This shows that the phenothiazine present in the feces following treatment did not destroy the viability of the parasite eggs but apparently prevented the earthworms from ingesting them. The data also show that administration of phenothiazine is ineffective for the removal of lungworms from swine.

31. *Limited Tests of Phenothiazine as an Anthelmintic in Goats.* PAUL D. HARWOOD AND JAMES E. GUTHRIE, Dr. Hess and Clark Laboratories, Ashland, Ohio.

Goat 22 received 25 grams of phenothiazine November 13, 1940. Fecal examination revealed two *Chabertia*, 61 *Oesophagostomum venulosum*, and 4 *O. columbianum* removed. Necroscopy 4 days after treatment revealed 9 *O. venulosum* and 3 *Chabertia*. Before treatment *Haemonchus* eggs were found in the feces, but no *Haemonchus* were recovered post-treatment. Goat 20 received 25 gms of phenothiazine February 11, 1941. Fecal examination revealed two *Oesophagostomum*

*columbianum* and one *Bunostomum* removed. By sampling the feces 30 *Trichostrongylus* (S.D.  $\pm$  3.75) were discovered. Necropsy on February 17 revealed 25 *Trichostrongylus* and 25 *Cooperia*. Goats 24 and 21 received 250 gms of phenothiazine each, and goat 23 received 100 gms November 13, 1940. Daily temperatures and haematocrit readings following treatment did not vary significantly. Milk yields of goats 24 and 21 dropped on November 16 to approximately 60 per cent of the pretreatment level, but were above pretreatment level November 18. Treatment January 21, of goat 21, with 1,000 gms of phenothiazine, produced similar effects. Goat 24 received 2,000 gms of phenothiazine April 2; the milk volume diminished by 94 per cent on April 5, but recovered to 90 per cent of the pretreatment level by April 18. Goat 24 was sluggish and appeared to suffer abdominal pains April 3; the symptoms diminished April 4. Twin kids born to goat 19, March 27, developed evenly and normally, although goat 19 received phenothiazine doses, each of 25 gms, March 18, April 15 and 25; and of 50 gms, April 30.

32. *Skin and Precipitin Tests for the Diagnosis of Trichina Infections in Grain-fed and in Garbage-fed Hogs.* L. A. SPINDLER AND JOHN L. AVERY, U. S. Bureau of Animal Industry.

In a large series of skin tests and a smaller series of precipitin tests made on hogs prior to slaughter in abattoirs, the tests often gave positive reactions in trichina-free hogs and sometimes failed to detect infections in hogs later shown to be trichinous. To test the effect of garbage feeding on the occurrence of non-specific skin and precipitin tests, skin and precipitin tests were made on 108 hogs experimentally fed garbage for 7 months and on 78 grain-fed hogs of comparable ages, sizes and breeds. Nine of the garbage-fed and 37 of the grain-fed hogs were experimentally infected with trichinae from 12 to 18 months prior to testing. The animals were kept outdoors under hog-lot conditions.

Of the infected, grain-fed hogs, positive reactions to precipitin tests were obtained in 81 per cent; skin tests were positive in 78 per cent; both tests were negative in 8 per cent. In the infected garbage-fed hogs, precipitin and skin tests were each positive in only 66 per cent; both tests were entirely negative in 22 per cent. In the uninfected grain-fed group, positive precipitin reactions were obtained in 23 per cent and positive skin tests in 13 per cent; both tests were positive in 7 per cent. In the uninfected garbage-fed hogs, positive precipitin tests occurred in 37 per cent and positive skin tests in 44 per cent; both tests were positive in 20 per cent. These data show that skin and precipitin tests for the diagnosis of trichina infections are less reliable in garbage-fed hogs than in hogs fed entirely on grain.

33. *Reactions in Garbage-fed and in Grain-fed Hogs to Intracutaneous Injections of Various Diluents.* JOHN L. AVERY, U. S. Bureau of Animal Industry.

In intracutaneous tests for the detection of trichina infections in swine, reactions to control injections of diluents occurred in as many as 12 per cent of various lots of hogs tested. Some of the diluents used are commonly employed in intracutaneous tests on human beings. To ascertain the effect of diluents alone when injected into swine, intracutaneous tests with several sterile solutions were made on 62 hogs fed garbage experimentally and on a comparable series of 50 grain-fed hogs. The reactions produced were in the form of either a solid pink, red, or purple color in the skin area injected, or an anemic wheal surrounded by a pink, red, or purple zone. Reactions to Coca's solution occurred in 39 per cent of the garbage-fed and 26 per cent of the grain-fed hogs. Coca's solution without phenol produced reactions in 51 per cent of the hogs fed garbage and in 43 per cent of those fed grain. Reactions to physiologic saline containing 0.5 per cent phenol occurred in 36 and 29 per cent of the two groups, respectively. A buffered saline solution produced reactions in 45 per cent of the garbage-fed and 8 per cent of the grain-fed hogs tested. These data show that reactions to intracutaneous injections of diluent occurred more frequently in the garbage-fed hogs than in those fed grain, and indicate that the diluents used contributed to the nonspecific reactions in swine.



34. *Precipitate Formation around Trichina Larvae in Sera from Trichina-infected, and Trichina-free Hogs.* L. A. SPINDLER, JOHN L. AVERY, AND HARRY E. ZIMMERMAN, JR., U. S. Bureau of Animal Industry.

Recently several investigators have reported the formation of precipitates around the mouth of decapsulated larvae of *Trichinella spiralis*, when the larvae were incubated in sera of trichina-infected rats, rabbits, guinea pigs, and humans. In 1933 the senior author incubated decapsulated trichina larvae in sera of 3 trichinous and 3 non-trichinous hogs. Precipitates formed around a small proportion of the larvae in sera from 2 of the 3 infected swine and also around some larvae in the sera of each of the uninfected hogs. Recent investigations by the present authors involved sera from 14 experimentally infected and 61 uninfected swine, the technique used being that described by Olivier-Gonzalez (1940), except that from 5 to 200 larvae were used per serum preparation. No precipitates formed in the case of larvae incubated in sera from 4 (29 per cent) of the infected hogs irrespective of the number of larvae used. In the remainder, from 1 to 71 per cent of the larvae involved (average 17 per cent) showed the formation of precipitates. Typical precipitates were observed in 1 to 73 per cent (average 7 per cent) of the larvae incubated in sera from 49 (88 per cent) of the uninfected hogs; sera from the remaining hogs failed to cause the formation of precipitates. Precipitates in sera from uninfected hogs appeared on microscopic examination to be identical with those in sera from infected hogs. These observations indicate that this test cannot be relied upon to detect with certainty trichina infections in swine.

35. *Experimental Infection of Pigs with the Swine Thorn-headed Worm, Macracanthorhynchus hirudinaceus.* KENNETH C. KATES, U. S. Bureau of Animal Industry.

Approximately 20 infective juvenile forms (acanthellas) of the swine thorn-headed worm were fed in one dose to each of 4 pigs (numbers 710, 706, 707, and 693), and a total of several hundred (estimated) acanthellas were fed to a fifth pig (number 777) in 4 successive doses at 4-week intervals. The infective stages in question were obtained from grubs, pupae and adults of the green June beetle, *Cotinis nitida*, that had been infected 2 to 3 months previously while in the final instar stage. The prepatent period of the parasite was about 2½ months and the patent period 6 to 9½ months, with the peak of egg production occurring 5 to 7 months after infection. At the peak of egg production each female worm produced approximately 400,000 eggs per day. The numbers of mature parasites recovered at necropsy from the pigs were respectively 2 females, 1 male; 1 female, 4 males; 6 females, 3 males; 1 female; and 6 females, 7 males. With the exception of pig 777 more nodules than worms were found in the jejunum of the host at necropsy, indicating that the parasites did not remain attached at one spot. This conclusion is based on the fact that except in pig 777 no acanthocephalids were passed in the feces during the course of the experiment. In the case of pig 777, all the females and one male were spontaneously eliminated by the host at various intervals from 6 months to 2 weeks prior to necropsy, 13 months after infection. At that time fewer nodules than worms were found, this indicating that the nodules disappeared following loss of the worms.

36. *The Duration of Infectivity of Nematode Parasites of Cattle on Florida Pasture with Observations on Resistance of Calves to Natural Re-infection with Haemonchus contortus.* DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama, LEONARD E. SWANSON AND TERRENCE J. DRAKE, University of Florida.

On April 1, 1941, six parasite-free yearlings were turned on a carpet grass pasture at Gainesville, Florida. This pasture was last grazed by parasitized cattle the previous fall (November 10, 1940). These yearlings were killed and examined for parasites, two each on the 35th, 91st, and 144th day after the beginning of the test. *Haemonchus contortus*, *H. similis*, *Ostertagia ostertagi*, *Trichostrongylus*

*axei*, *Cooperia punctata*, *C. pectinata*, *Oesophagostomum radiatum* and *Trichuris* sp. were recovered from these yearlings collectively but *Bunostomum phlebotomum* and *Dictyocaulus viviparus*, also present in cattle on the pasture the previous November, were not found. The free-living stages of the latter species were apparently unable to remain infective for the period of nearly five months during which the pasture had been vacant. (Temperature, 24° to 85° F, rainfall 18.05 inches.) Judging from the number of worms recovered, *H. contortus* and *O. radiatum* were by far the most successful survivors. One each of the first two pairs of yearlings had been previously infected experimentally with *H. contortus* at Auburn, Alabama, but had eliminated these worms some time before going on pasture. That these animals were resistant to natural reinfection with this parasite was indicated by the failure of *H. contortus* to develop in them, whereas the other yearlings acquired large numbers of this species.

37. *The Occurrence of Mites in Pinnipeds, Including a New Species from the California Sea-Lion*, *Zalophus californianus*. WILLIS H. DOETSCHMAN, University of Southern California and The Biological Research Institute, Zoological Gardens of San Diego.

A number of sea mammals, belonging to the suborder Pinnipedia, were examined at autopsy at the San Diego Zoo to determine the prevalence of mites belonging to the genus *Halarachne* Allman, 1847. Permanent mounts were made by a modified technique of Ewing's combination clearing-mounting media. The main changes in Ewing's technique were the addition of basic fuchsin stain and the use of less chloral hydrate and more glycerol. A total of 74 animals were studied including 55 California sea-lions, *Zalophus californianus*; 6 Stellar's sea-lions, *Eumetopias jubata*; 11 California harbor seals, *Phoca richardii*; and 2 elephant seals, *Mirounga angustirostris*. Infection of the lungs was found in 60.8 per cent and of the nasal passages in 21.8 per cent of these animals; a total of 70 per cent were infected. *H. zalophi* Oudemans, was found in the nasal passages of Stellar's sea-lion (a new host record) and the California sea-lion. *H. miroungae* Ferris, was taken from the nasal passages of the California harbor seal (a new host record) as well as the elephant seal. Male specimens of this species, previously unreported, were also collected.

A new species was recovered from the bronchial passages of the California sea-lion. This species is smaller than any previously described. The average size of the females is 920  $\mu$ ; males, 660  $\mu$ . The dorsal plate is oval and contains 6 pairs of setae in both sexes. Morphology of the mouth parts of this species is distinctly different from species previously reported. A more detailed description of this mite and a discussion of the validity of the existing species of *Halarachne* will appear in another paper.

38. *Observations on the Duration of an Infection of Hymenolepis nana in Man*. WILLIAM HUGH HEADLEE, Purdue University.

In 1933 a male individual 26 years of age was found to be infected with *Hymenolepis nana*. From January, 1933, to August, 1940, thirty-four stools from this person were examined and each was found to be positive for that parasite. The infection was light when discovered, and continued to be light until June, 1938. At that time the infection became markedly heavier, and then gradually decreased in intensity until it reached its former level after six months. It then became necessary on occasion to use concentrate methods in order to detect the infection. Dilution egg-counts were made on eight stools, the number of ova detected ranging from 200 to 7,800 per gram B.F. At the time the tapeworm infection increased markedly, a heavy infection of *Chilomastix mesnili* was found, and it persisted for a month. The increase in the intensity of the tapeworm infection at that time may have resulted from a disturbed balance due to the presence of *C. mesnili* in large numbers. Other parasites frequently found were the amoeba, *Endolimax nana*, and the yeast, *Blastocystis hominis*. The infection of *H. nana* was terminated in August, 1940, by treatment with hexylresorcinol. Four stools examined since that time, over a

period of more than a year, have been negative. Reinfection was guarded against by the practice of rigid sanitary habits. It is considered that direct reinfection did not likely occur and that the life-span of this cestode is considerably greater than formerly believed, or that internal autoinfection may have occurred.

39. *The Effect of Host Age on the Number of Trichinella spiralis Recovered from Rats during the Early Period of Infection.* L. O. NOLF AND HERMAN ZAIMAN, State University of Iowa.

Sixty-eight rats belonging to three age groups were used in five experiments to study the effect of host age on the intestinal phase of trichinosis in rats. Group A consisted of 28 rats ranging in age from 3 to 12 months. Group B consisted of 22 five-week-old rats. Group C consisted of 18 unweaned rats between 8 and 15 days old. Each rat received 2,000 *T. spiralis* larvae per os. With only two exceptions, recoveries were made between 36 and 86 hours after infection. The two exceptions were a 15-day-old rat and a 12-month-old rat which were autopsied eight and one-half days after infection. For statistical analysis, the data from all rats in the same group were considered together. The lowest average number of worms, 1,042 (S.D.=246), was recovered from the unweaned rats, Group C. The next highest average number, 1,554 (S.D.=260), was recovered from Group A, the 3 to 12-month-old rats. The highest average recovery, 1,777 (S.D.=175), was made from the five-week-old rats, Group B.

The difference between the averages of Groups A and C is 512. It is 6.6 times the standard error of the difference of the means. The difference between the averages of Groups B and A is 223. It is 3.6 times the standard error of the difference of the means. The difference between the averages of Groups B and C is 735. It is 10.4 times the standard error of the difference of the means.

40. *The Effects of Immune Serum upon the Larvae of Trichinella spiralis in Vitro.* EDWARD P. OFFUTT, JR., University of Rochester.

In repeated experiments, no significant effect has been demonstrated on the infectivity for rats of *Trichinella spiralis* larvae following their exposure to immune rabbit serum for periods as long as from three to five days. Such larvae showed approximately the same infectivity as larvae exposed to normal rabbit serum under similar conditions. Infectivity was measured by counting the number of larvae that were able to develop to the adult stage. In one experiment, the productivity of the resultant worms was determined by counting the number of larvae recovered upon digestion of the rats a month later. No significant effect was observed.

All immune sera used in the present experiments possessed precipitin titers of at least 1:10,000. Precipitates were observed to form at the anterior ends and along the bodies of larvae incubated in immune serum at 37° C, but the death rate of these larvae was not markedly different from that of larvae incubated in normal serum. A few larvae remained alive for as long as three weeks in both immune and normal sera.

41. *Attempted Passive Transfer to Rats and Mice of Immunity to Trichinella spiralis.* O. R. MCCOY AND FRANKLYN F. BOND, University of Rochester.

Various attempts to transfer immunity to rats and mice by the intraperitoneal injection of serum from rats and rabbits which had survived repeated feedings with larvae of *Trichinella spiralis* have failed to demonstrate the passive transfer of any significant degree of increased resistance. In one typical experiment, serum pooled from 48 highly resistant rats was injected into 15 young adult rats in amounts totaling 6 cc per 100 gm of body weight. Seven rats killed within 8 days after a test dose of 1,500 *Trichinella* larvae contained approximately the same number of worms in the intestine as was found in control animals. Also, the average number of larvae recovered by digestion from the muscles of the remaining 8 rats, which were killed after 6 weeks, was not significantly different from the number recovered from 8 control animals.

Four experiments were performed in which groups of 10 mice were injected with immune rabbit sera having precipitin titers of at least 1:12,500. Each mouse received approximately 10 cc of serum per 100 gm of body weight. When fed a test dose of 300 *Trichinella* larvae, the mice injected with immune serum showed, on the average, about the same amount of intestinal and muscle infection as did comparable groups of control mice which either had received normal rabbit serum or were untreated. These findings fail to confirm the experiments of Culbertson and Kaplan (1938, *Parasitology* 30: 156-166) which reported increased resistance in mice injected with immune rabbit serum.

42. *The Protective Action of Normal Sheep Serum against Infections of Trypanosoma duttoni in the Mouse.* WILLIAM H. TALIAFERRO AND YELENA PAVLINOVA OLSEN, University of Chicago.

The protective property of sheep serum against *T. duttoni* in mice described by Thiroux has been amply confirmed. Complete or partial protection may result from doses as low as 0.1 cc sheep serum per 20 gm mouse. Zone phenomena are frequent in titrating serums with graded doses. The anti-*duttoni* property is of variable occurrence in sheep and varies in titer in the same animal at different times. No satisfactory explanation for most of this variability has been found. Evidence from sheep of different ages and serial tests during two years on one mother and her lamb indicate that the protective property is present in the mother, but absent in the newborn lamb and does not appear in the lamb's blood until after a year. The titer of the protective property is only diminished just before death after severe liver injury due to (a) poisoning with carbon tetrachloride, (b) pregnancy disease and (c) partial occlusion of the vena cava.

43. *The Trypanocidal Action of Sheep Serum on Trypanosoma duttoni.* WILLIAM H. TALIAFERRO AND LUCY GRAVES TALIAFERRO, University of Chicago.

In the mouse, the protective property of sheep serum against infection with *T. duttoni* is slightly decreased after splenectomy and markedly decreased when splenectomy is combined with blockade by repeated doses of India ink or sheep serum (exhaustion experiments). In vitro, sheep serum is trypanocidal to *T. duttoni*. Although the results are not entirely consistent, they indicate that the property behaves like an antibody since it is absorbed by the parasites and is inactivated by heating at 56° C for 20 minutes and reactivated by the addition of fresh guinea pig serum.

44. *Quantitative Relationships in Immunity to Hookworm.* G. F. OTTO, Johns Hopkins University.

Single doses of 1,200 to 1,400 infective larvae of the dog hookworm, *Ancylostoma caninum*, induced immunity in dogs sufficiently powerful to protect them from subsequent infections of 50,000 larvae. Single doses of 300 larvae, however, were not sufficient to protect the animals from 50,000 larvae. It seems likely, however, that some degree of immunity was induced by 300 larvae since the sera of such animals, in some cases, formed precipitates with living larvae in vitro. A similar precipitin reaction was observed with *Necator americanus* larvae in sera of infected humans. Preliminary studies, however, indicate that the reaction is not qualitatively specific since some canine immune sera reacted with *N. americanus* larvae and some sera of infected humans reacted with *A. caninum* larvae.

45. *Natural Resistance to Helminthic Infections.* JAMES E. ACKERT, Kansas State College.

Presidential address.

46. *A Case of Intestinal Myiasis.* MARCUS W. LYON, South Bend Clinic, AND JOHN D. MIZELLE, University of Notre Dame.

A twenty-three-year-old woman of South Bend, Indiana, reported to her physician that she had passed "worms" in her stools for the interval of approximately one

month. A specimen examined in August, 1941, by the senior author revealed that the organisms in question were fly larvae (*Lucilia sericata*). The patient reported no intestinal symptoms; the stool examined was normal in shape and color; and the larvae present were more toward the outside of the fecal mass than otherwise. Three years prior to this diagnosis the patient complained of a burning sensation during urination but it is thought that this probably has no connection with the *Lucilia sericata* infection. However, Chandler has reported (1941, J. Parasitol. 27: 465), a case of urinary myiasis with symptoms of a similar nature. It is not known how the maggots gained access to the digestive tract of the patient. Her house was unscreened and she complained of the large number of flies therein.

The authors are grateful to Dr. W. B. Herms for specific identification of the larvae.

47. *Hexamita in the Blood of a Mouse*. JUSTIN ANDREWS AND MADGE REYNOLDS, Georgia Department of Public Health.

The specimen shown is a thin blood film made from the lung-blood of an autopsied mouse, one of three inoculated intraperitoneally with human blood from a suspected case of relapsing fever. Two remained healthy and never gave evidence of blood parasitism. The twelfth day after inoculation, the third mouse showed progressive signs of sickness—sluggishness, rough coat, loss of weight. Films of tail blood diluted with sterile physiological saline were examined for spirochetes at daily intervals commencing the fourth day after inoculation. On the 12th, 13th, 14th and 15th days, unidentified organisms called flagellates, because of their characteristic, jerky movements, were seen in both bright- and dark-field preparations. These were present in small numbers and could not be found in Giemsa-stained thin films. On the 16th day after inoculation, the mouse died and was autopsied six hours thereafter. Thin smears were prepared from the lung, liver, spleen and kidney. On each of these, stained with Giemsa's stain, organisms which appear to belong to the genus *Hexamita* (*H. muris*?) were found. They were most plentiful in the lung-blood. There was no evidence of contamination with intestinal contents in any of the blood smears. No attempt was made to transfer these organisms to other mice.

Thin blood films were examined from 116 other members of the mouse colony but no *Hexamita* was found. Twelve of these were autopsied and smears of upper and large intestinal contents in physiological saline were examined. An abundant colonization of *Hexamita* throughout the intestine was found in a single case. According to Wenyon (1926), *Hexamita* has been found in the blood of cold-blooded vertebrates (frogs and tortoises). This appears to be the first record of its presence in the blood of a mammal.

48. *Two Unusual Trematodes*. H. W. MANTER, University of Nebraska.

Demonstration of black trematodes (*Campula* species) from the liver of a porpoise (*Tursiops gillii*) from the Pacific. Although Poirier (1886) described *Campula rochebruni* as "couleur noirâtre," he attributed this color "au vitellogène très développé sur toute la longueur du corps." In my specimens a very persistent black pigment is scattered in granules throughout the parenchyma. Living specimens were very black.

A lepecreadid trematode from a marine fish (*Epinephelus morio*) from Tortugas, Florida, has the genital pore median and directly posterior to the acetabulum. It represents a genus near *Anallocreadium*.

49. *Two Species of Porrocaecum (Nematoda) from the Robin*. J. DAN WEBSTER, Rice Institute.

More than 30 individual worms of this genus were taken from 7 out of 19 American robins (*Turdus migratorius*) examined at Ithaca, New York. Five specimens are *Porrocaecum ensicaudatum*, which has previously been reported in North America only from the mockingbird (*Mimus polyglottus*); the rest belong to a new species of *Porrocaecum* which is most nearly related to *P. cheni* of the Chinese black-bird (*Turdus mandarinus*).



50. *The Genus Gnathostoma in North America.* ASA C. CHANDLER, Rice Institute.

Leidy, 1858, reported a gnathostome from the stomach of a mink; this species is now regarded as identical with *Gnathostoma spinigerum* of Old World carnivores. The opossum harbors a large species, *G. didelphis*, which has been reported from Louisiana and Texas. A new species, smaller than *didelphis*, has now been found in a high percentage of raccoons in east Texas. The body scales of these gnathostomes present the best differential characters. They differ in area of body covered, general size and shape, number of points on scales at different levels of the body, and most strikingly in their method of dwindling away posteriorly. In *spinigerum* the scales become single, widely scattered spines about 25 to 40  $\mu$  long; in *didelphis* they become minute scattered spines only about 10  $\mu$  long, and in the gnathostome of the raccoon they fuse into transverse serrated ridges.

51. *Demonstration of a New Parasitic Nematode from a Water Scavenger Beetle.* A. C. TODD, University of Nebraska.

Thirty-one adult female and 26 adult male nematodes were recovered from the water scavenger beetle, *Tropisternus nimbatus* Say. (The beetles were obtained through the courtesy of Mr. M. J. Harbaugh.) The new nematodes appear to be congeneric with *Oxyuris* (*Helicotherix*) *hydroi* described by Galeb in 1878 from *Hydrous caraboides*. Interesting features of the worms are the prominence of the anterior lip of the vulva and the coiled filament which surrounds the egg.

52. *The Host-Parasite Phylogenetic Complex: The Criteria and Their Application to the Opalinid-Anuran Complex.* JOHN LUTHER MOHR, University of California.

The proof of parallel evolution of parasite and host groups rests upon positive identification of parasite and host species. It is therefore desirable to use groups with obvious specific characters. Materials must be in good condition. Samples must be numerous and scattered evenly taxonomically, ecologically, and geographically. Other possible hosts must be sought in the same ecological niche and in animals related to known host groups. If on the basis of population surveys there seems to be evidence of host-parasite specificity, one should investigate limiting factors and the possibilities of making experimental transfers of parasites to hosts closely and distantly related to the wild hosts.

With a body of positive evidence of rigid host specificity from both survey and experimental sources, one may examine the host and parasite series to determine which forms are primitive and which, derivative. If this can be determined satisfactorily in both series, he should see whether primitive parasites inhabit primitive hosts, etc. If there is such coincidence, one may conclude that the groups probably evolved *pari passu*.

Studies on the opalinid-anuran complex have been deficient because of poorly preserved materials, insufficient numbers, and poorly distributed sampling. Experimental studies have been inadequate and opalinid and anuran phylogenies have been carelessly drawn (by the workers on the parasites). A phylogenetic parallelism has not been demonstrated for this complex.

53. *Some Observations on Histomonas from Wild Pheasants and Domestic Fowls.* D. H. WENRICH, University of Pennsylvania.

Measurements of 200 histomonads on prepared slides from ceca of two wild ring-necked pheasants (*Phasianus torquatus*) gave a range of diameter from 9.5 to 19.5 microns with an average of 13.9 microns. Larger individuals up to 27 microns in diameter were seen. Most showed 4 flagella arising from a pair of basal granules; a rhizostyle extended inwards along one side of the nucleus. A hyaline ectosarc was characteristic. About 12 per cent of histomonads from one pheasant showed a tubular or cylindrical extension from the surface, differentiated from the endosarc and covered by a layer of ectosarc and the surface membrane. Extensions

varied in width from 1 to 4 microns, and in length up to more than the cell diameter often with internal components which were equally long. They occurred at any point on the periphery and usually had one or more bacteria or other small bodies in a concavity at the outer end. Bacteria and filaments were ingested without the formation of such extensions. On slides from domestic fowls kindly supplied by Dr. E. E. Tyzzer, similar differentiations were seen. Possible interpretations: 1) a peculiar type of pseudopodium; 2) an attachment organelle (cf. the rostellum of oxymonad flagellates); 3) an ingestion tube.

On Tyzzer's slides his description of *Histomonas meleagridis* was largely confirmed, most of the individuals being considerably smaller than those in the pheasants and each bearing one flagellum or none. Tyzzer's "giants" with 4 flagella apparently correspond to the majority of individuals seen from the pheasants.

54. *The Pathogenicity for Kittens of Endamoeba histolytica from a Human Case of Acute Amoebic Dysentery.* CHARLES W. REES AND LUCY V. REARDON, National Institute of Health.

No abstract received.

55. *Pantothenic Acid and Eimeria nieschulzi Infection in the White Rat.* ELERY R. BECKER, Iowa State College.

Previously reported experiments have disclosed that when tests of the coccidium-growth-promoting potency of certain pure synthetic vitamins were made by employing them as supplements to a particular standard ration low in vitamin content, vitamin B<sub>1</sub> moderately depressed oöcyst counts, vitamin B<sub>6</sub> markedly increased them, while these two vitamins together produced an exceedingly marked depression of the numbers of oöcysts eliminated. The new studies are concerned with the effects of crystalline synthetic pantothenic acid supplements to a standard ration rather similar to that previously used. Pantothenic acid supplement alone enhances moderately the numbers of oöcysts eliminated. When, however, the control series of rats receives both the standard ration and pantothenic acid, test rats receiving the same regimen plus vitamins B<sub>1</sub> and B<sub>6</sub> do not, apparently, eliminate fewer oöcysts during the infection. Pantothenic acid in some way interferes with the coccidium-growth-depressing action of vitamin B<sub>1</sub> and vitamin B<sub>6</sub> fed together.

56. *Transmission of the Liver Coccidium, Eimeria stiedae, from the Domestic to the Cottontail Rabbit.* HARRY A. JANKIEWICZ, University of Southern California.

A preliminary examination of 5 young cottontails, *Sylvilagus audubonii valli-cola*, from the San Joaquin area demonstrated no liver coccidiosis. Three cottontails were then orally inoculated with sporulated oöcysts of *E. stiedae* from domestic rabbits. Two received 7,500 oöcysts each on one day and 4,500 oöcysts on the following day. The third received 8,000 oöcysts on one day and a similar dosage two days later. Oöcysts of *E. stiedae* first appeared in the feces 18 days after inoculation. Upon autopsy 18 to 24 days after inoculation, the gall bladders and bile ducts of all three cottontails harbored oöcysts of *E. stiedae* morphologically and physiologically identical with those originally employed. One liver was very lightly infected the second more heavily infected, and the third very heavily infected. Two cottontails used as controls remained negative. Sporulated oöcysts from the heavily infected liver were fed to another cottontail on three successive days, 2,300 given daily. Eighteen days later, oöcysts of *E. stiedae* appeared in the feces and autopsy on the 20th day revealed a small number of oöcysts of *E. stiedae* in the bile fluid. The pathological findings are similar in both domestic and cottontail rabbits. Four squirrels, *Citellus beechyi*, given 8,000 oöcysts of *E. stiedae* from the domestic rabbit remained negative. Three of those inoculated mechanically carried oöcysts of *E. stiedae* through their digestive tracts 1 to 4 days after inoculation.

57. *Life Cycles of Four Species of Intestinal Coccidia of the Domestic Rabbit.* ROBERT L. RUTHERFORD AND JOHN F. KESSEL, University of Southern California.

The endogenous cycles of four intestinal species of *Eimeria*, i.e., *E. irresidua*, *E. magna*, *E. media*, and *E. perforans* have been studied in the domestic rabbit. Each species produces two types of merozoites. They are designated as Type "A" and Type "B," each producing a first and second generation of merozoites. It is possible that the first generation of Type "A" merozoites gives rise to microgametocytes or a second generation of merozoites, while Type "B" gives rise to macrogametocytes or a second generation of Type "B" merozoites. The four species require the following lengths of time for completion of their endogenous cycles: *E. irresidua*, 9 to 10 days; *E. magna*, 7 days; *E. media*, 6 days, and *E. perforans*, 5 days.

58. *A Study of the Paroxysms Resulting from Induced Infections of Plasmodium vivax.* G. ROBERT COATNEY AND MARTIN D. YOUNG, U. S. Public Health Service.

Three hundred and thirty-eight paroxysms were studied in 21 white male neurosyphilitics infected with the St. Elizabeth strain of *Plasmodium vivax*. Chills occurred in only 201 (59 per cent) of the paroxysms. The absence of chills in 41 per cent of these attacks, together with the fact that chills do not occur in 76 per cent of the quartan malaria paroxysms in Negro paretics, indicates that the term "chills and fever" inadequately describes a malarial paroxysm. The temperature was already elevated at the start of the chill, averaging 100.6° F. The average length of the chill was 39 minutes and the average temperature rise during this phenomenon was 2.3° F. The average measurements for the 338 paroxysms were: time from onset of fever (100° F) to fever-peak, 3 hours, 52 minutes; time from fever-peak to end of fever, 6 hours, 18 minutes; fever-peak, 104.8° F. The duration of the temperature of 100° F and above averaged 10 hours, 10 minutes per paroxysm. Using this as a measure, the total hours of fever experienced by a white parietic undergoing malaria therapy with the St. Elizabeth strain of *P. vivax* can be estimated by multiplying the number of paroxysms by ten.

The 201 chill-accompanied paroxysms were compared with the 137 paroxysms without chills. The fever-peak average was 0.7° F higher in the chill-group and this difference was found to be statistically significant (6.26). Also, the average duration of the fever was 2 hours and 2 minutes shorter in the chill-accompanied group, but this difference is considered of doubtful statistical significance. The average rate of fever rise was 3.3 times faster during the chill (1° F in 17 minutes), than during any other period of fever rise (1° F in 56 minutes). It seems that the occurrence of a chill is directly responsible for the more rapid temperature rise and the higher peak temperature in the chill-accompanied paroxysm.

59. *Saurian Malaria.* CLAY G. HUFF, University of Chicago.

Natural infections of *Plasmodium* were found in numerous lizards of the species *Sceloporus f. ferrariperesi* and in occasional specimens of *S. m. microlepidotus* and *Basiliscus vittatus* from Mexico, and commonly in *Sceloporus u. undulatus* from Florida. The latter has been transferred by blood to collared lizards (*Crotaphytus collaris*), leopard lizards (*C. wislizenii*) and anoles (*Anolis carolinensis*). Other species (*Cnemidophorus t. tessellatus*, *Phrynosoma coronatum* and *P. orbiculare*) have been refractory to infection. *Culex pipiens* and *Aedes aegypti* fed readily on *S. undulatus* and one nearly mature oöcyst was found on the stomach of an *A. aegypti* which had fed on an infected lizard. No evidence for the existence of exoerythrocytic schizogony was discovered in any of the natural or experimental infections. While it is believed that more than one species are represented in our laboratory infections, no identification or description will be made until extensive studies have been completed.

60. *Blood-cell Counts in Plasmodium durae Infections.* CARLTON M. HERMAN AND JEANNE YOUNG, Los Angeles Wildlife Disease Research Station.

Because of the wide variation in blood-cell counts in normal birds, a single low R.B.C. count cannot be considered diagnostic of infection. In normal control

turkeys the R.B.C. counts varied from 1,990,000 to 2,570,000 per cmm of blood from one bird and from 1,448,000 to 2,984,000 from another. The white cell counts varied from 20,800 to 29,500 per cmm of blood and from 13,800 to 34,800, respectively, from the same two birds. In the six turkeys inoculated with *Plasmodium durnae* the lowest count obtained was 1,248,000 R.B.C. Examinations were made at intervals of two and three days. In general the R.B.C. counts tended to drop with the appearance of over 100 parasites per 10,000 R.B.C. and remain at an average lower level until death or recovery. No apparent correlation was evident between parasite counts and individual R.B.C. counts, although the lower trend of the R.B.C. counts was much more pronounced and sustained in the infections with greater numbers of parasites. The changes in the W.B.C. counts were usually more marked, tending to be higher during infection and reaching 78,800 in one bird just before the peak of the patent period; however, the trend phenomenon was not as pronounced. Similar results were obtained with *P. durnae* infections in Pekin ducks and in California Valley quail.

61. *The Anti-malarial Effect of Acranil.* WENDELL D. GINGRICH AND ROLLIN S. FILLMORE, School of Medicine, University of Texas.

Acranil, chemically related to atabrine, was tested prophylactically and curatively with infections of *Plasmodium cathemerium* in canaries. A series of 30 birds was inoculated intramuscularly with pooled infected blood. In 5 of these which had received 2 mg in 0.1 ml 5 per cent gelatin daily by stomach tube for 5 days, previous to inoculation, the incubation period averaged 10.4 days; whereas the incubation period averaged 4 days in the 5 controls. The blood examinations became negative in 3.4 days in 5 birds treated with 2 mg acranil daily for 5 days after the initial acute infection had developed, and in 4 days in 5 birds similarly treated with atabrine. In 5 birds of the same series the daily administration of 2 mg of acranil for 10 days immediately following inoculation delayed the appearance of parasites in the blood for 20.6 days, whereas similar treatment with atabrine delayed the appearance of parasites for more than 25 days.

62. *Reservoir Hosts of Chagas' Disease in the State of Texas.* A. PACK-CHANIAN, School of Medicine, University of Texas.

A number of small mammals were collected in the state of Texas (armadillos, mice, and wood-rats around Three Rivers, Texas; opossum around Austin, Texas). Of these one nine-banded armadillo (*Dasyfus novemcinctus*); 8 opossums (*Didelphys virginiana*), two house mice (*Mus musculus*), 32 wood-rats (*Neotoma micropus micropus*) were found naturally infected with *Trypanosoma cruzi*.

63. *The Occurrence of Heterodoxus longitarsus Piaget (Mallophaga: Boopinae) on Dogs in Mississippi.* J. W. WARD, Mississippi State College.

*Heterodoxus longitarsus* Piaget, a biting louse, was found to be present on 7 out of 40 dogs examined at this College during the past 10 years. These interesting specimens belong to the family Menoponidae, the most of which infest birds. Members of one small sub-family, the Boopinae, originally were parasites infesting marsupials in Australia. *H. longitarsus* has been reported from the dog twice from Africa (one of which is a personal communication from Dr. Rene du Toit), once from Formosa, once from San Francisco, and once from Oklahoma. It is interesting to note that this parasite, which had as its original host a mammal that is at the lowest end of the phylogenetic scale and was once restricted to a small section of the world, now seems to have established itself on mammals near the other end of the phylogenetic scale and has become distributed throughout the world.

64. *Mechanical Transmission of Shope Rabbit Fibroma by Certain Haematophagous Bugs.* CORNELIUS B. PHILIP, Hamilton, Montana.

Mechanical transfer of fibroma virus between a donor domestic hare, and 2 test hares each by interrupted feedings and injection of 3 species of Triatomidae was

successful as judged by resistance of test animals to subsequent challenge doses of controlled myxoma virus. Three of these had shown small local lesions following test, while one bitten animal was without any visible reaction at the site of attack. The donor was used on the fourth and seventh days after it was infected. On the other hand, animals tested by injection and feeding of "incubated" insects succumbed in the usual way when infected with myxoma virus later.

65. *Oxidation-reduction Potentials in Relation to the Cultivation of Endamoeba histolytica.* LEON JACOBS, National Institute of Health.

Potential-time curves have been obtained on pure cultures of each of the following bacteria: *Leptotrichia buccalis*, *Clostridium welchii*, *Streptococcus hemolyticus*, *Bacillus subtilis* and *Bacterium coronafaciens*. A rapid drop in the potential of the medium to an average minimum of -336 mv occurs with *L. buccalis* and to -500 mv with *Cl. welchii*, levels which are reached within the first 10 hours. The potentials hover around the minimum for 24 to 40 hours, then exhibit a more or less gradual return to positive levels. A minimum of -114 to -150 mv is attained after 25 hours of cultivation of *S. hemolyticus* and *B. subtilis* and after 72 hours with *B. coronafaciens*; this potential is maintained for up to 3 weeks.

Cultures of *E. histolytica* with *L. buccalis* or *Cl. welchii* show abundant growth but require transplants after 4 or 5 days for best results, while with the other organisms the amoebae persist in the same tube for up to 3 weeks. Although excystation and growth of amoebae will take place at a potential of -114 to -150 mv, growth is more prolific at -300 to -500 mv. Also, the longevity of amoeba cultures is correlated with the maintenance of reducing potentials. These data explain the success of Snyder and Meleney in securing excystation with cysteine HCl, which produces a potential of -160 to -200 mv in the concentration used by them. The data also indicate that the cultivation of *E. histolytica* without bacteria will require control of the oxidation-reduction potentials of the medium, a heretofore neglected factor.

66. *Indicanuria and the Vitamin A Absorption Test in Giardiasis.* C. P. KAT-SAMPES, W. A. PHILLIPS, A. B. MCCOORD AND S. T. HARRIS, University of Rochester.

In a series of 15 cases showing *Giardia* cysts in the stool associated with mild or severe upper abdominal pain, 12, or 80 per cent, had indicanuria. In 6 of the 12 cases, a duodenal drainage was also done and in every case the trophozoites of *Giardia lamblia* were found in the duodenal contents. The presence of detoxified indole (indican) in the urine of such cases suggests the possibility of interference by the parasite with protein absorption in the upper intestinal tract.

In 3 cases so far studied, a marked decrease in the rate and degree of fat absorption was detected by the vitamin A absorption test. Ten to 14 days after treatment with atabrine, when the stool and duodenal contents showed no *Giardia*, in all cases a moderate but definite improvement in fat absorption was found. In one case, a repeat test 16 days later showed a very marked improvement in fat absorption. In another of the 3 cases, the cysts reappeared in the stool associated with indicanuria and abdominal pain 5 months after the previous atabrine treatment, and the absorption test results remained well below the normal average. The results with this test so far appear to bear out the contention of Veghelyi (1940) that giardiasis interferes with fat absorption.

67. *Incidence of Protozoan Infection amongst the Personnel of a Large General Hospital with Special Regard to Incidence according to Race and Occupation.* MAURICE M. ROTHMAN AND MARION LASKEY, University of Pennsylvania.

A protozoan survey was made of the personnel and staff at the Graduate Hospital of the University of Pennsylvania. Included amongst them were the internes, nurses, nurses' aides, porters, orderlies, maids, elevator operators, and those from the diet kitchen, laboratory, record room, administration office and x-ray depart-



ment. A questionnaire regarding the individual's travels, pertinent past medical history, and present symptoms if any, accompanied each specimen submitted. Each of the authors independently examined all of the material by fresh wet preparations and stained slides. A total of 307 individuals submitted specimens. They were divided into two main race groups, the white and the colored. The incidence of infection as a whole is recorded as well as the incidence of infection within the individual groups.

68. *Survival Time of Intravaginally Implanted Trichomonas hominis*. ROBERT M. STABLER, University of Pennsylvania, AND L. G. FEO, Jefferson Medical College Hospital.

*Trichomonas hominis*, cultured from the human intestine, could not be recovered from the vaginas of 25 women 48 hours after inoculation. Of these, 10 were negative for *T. vaginalis* and 15 were positive. Seven women (two negative, five positive for *T. vaginalis*) were examined after a 24-hour interval. One (positive for *T. vaginalis*) was still positive for *T. hominis*. Of those examined at shorter intervals, two at six hours gave one positive and one negative; seven at four hours gave two positive and five negative; two at two hours were both positive; at one and one-half hours, three were negative and five positive; at one hour four were negative and seven positive; and of two examined at one-half hour, one was positive, one negative. Two of the seven positive at one hour were negative at one and one-half hours. One woman, positive at one and one-half hours, proved negative in a subsequent four-hour experiment. The one person positive for *T. hominis* at 24 hours, was negative when tried later in the 48 hour series. It is concluded that *T. hominis* will not survive in some human vaginas even for one-half hour; that in others it may live for as long as 24 hours; that it does not survive intravaginally as long as 48 hours. The bacterial and pH conditions associated with these cases will be reported later in detail.

69. *Notes on a Microsporidian Parasite, Duboscqia sp., of Reticulitermes of Maryland*. RICHARD R. KUDO, University of Illinois.

Of 330 workers of *Reticulitermes* sp., collected near Solomons Island, Maryland, and studied at the Chesapeake Biological Laboratory in July and August, 1941, 21 individuals were found to be infected by a microsporidian. The organisms occur in the hypertrophied adipose tissue or "cysts" which are attached to the anterior region of the mid-intestine, and suspended freely in the hemolymph. The number of such cysts present in a single host varies from one to eleven. They are opaque white and spherical to ellipsoidal, varying in size from 200  $\mu$  in diameter up to 625 by 375  $\mu$ . The cyst is filled with young and old sporonts which contain developing sporoblasts or spores. The developing sporont contains 16 sporoblasts that develop into 16 spores. The mature sporonts are subspherical to ovoid, and measure 11-14  $\mu$  by 8.6-10  $\mu$ . The sporont membrane is delicate, but distinct and holds the 16 spores in a group long after spores become fully-formed. The spore is ellipsoidal and measures 4.3-5.9  $\mu$  by 2.2-3  $\mu$  in life. Extruded polar filaments are 85-120  $\mu$  long. Feulgen demonstrates a single nucleus in the sporoplasm. Seeing a similar microsporidian in the body cavity of *Reticulitermes lucifugus* of France, Pérez (1908) established a new genus and species, *Duboscqia legeri* for it. This is one of a few true protozoan parasites, and, as far as the present writer is aware, the third record of microsporidian infection in the termite.

70. *The Fate of Some Species of Schistosome Cercariae in Chick Embryos*. STERLING BRACKETT AND ALBERT J. BECKMANN, University of North Carolina, and the Biological Station of the University of Michigan.

Chick embryos were tried as a possible culture medium for the development of some of the dermatitis-producing schistosome cercariae (*C. elvae*, *C. stagnicolae*, and *C. physellae*) whose life cycles have thus far remained unsolved. Chick embryos were chosen since it is common experience, in virus and bacteria experimentation,

that they constitute a versatile and non-specific medium. Furthermore, McCoy (1936, J. Parasitol. 22: 54-59) obtained development of the larvae of *Trichinella spiralis* in chick embryos. The cercariae were washed in a continuous flow of sterile water in a closed system after which they were introduced aseptically onto the chick embryos. The cercariae were never completely freed of bacteria as shown by bacteriological controls but 18 embryos lived 3 days or more after inoculation, and 6 hatched. Eight embryos were sacrificed and these plus 15 that died were examined for developing schistosomes but all were negative. Observations indicated that the cercariae did not penetrate the tissues of the embryo. Cercariae were then placed in saline extracts of minced chick embryos of various ages. The cercariae remained unaffected (retained the free-living behavior) for longer than two hours in extracts of embryos not over 17 days old. In extracts of chick embryos 18 days old or older the cercariae were inactivated and killed in less than two hours. It is suggested that chick embryos less than 18 days old do not possess substances necessary to stimulate the development of the cercariae but that on about the 18th day a cercaricidal substance develops which is capable of killing the cercariae rapidly.

71. *Studies on Host-Parasite Reactions. VI. An Hypothesis to Account for Pigmented Metacercarial Cysts in Fish.* GEORGE W. HUNTER, III, Wesleyan University.

Several years ago a program centering around a study of larval parasite-host reactions in fish was initiated with the hope of explaining the underlying differences noted in host reactions between such tissue penetrating groups as the Strigeidae, Clinostomidae and Heterophyidae. After the life cycles were known the next step involved an histological study of the cysts in their various locations. In this way the metacercarial cysts of *C. marginatum*, *Posthodiplostomum minimum*, *Urulifer ambloplitis*, and *Crassiphiala bulboglossa* have been described, using special connective tissue stains. The cyst of *Cryptocotyle lingua* is now being examined.

Recently the relationship between the parasite, *C. lingua*, and its metacercarial host, the cunner, *T. adspersus*, has been studied with the hope of determining the mechanism involved in the production and deposition of pigment in the melanin bearing cells that surround the parasite cyst. Cyst and normal melanophores were compared by various physiological means, while the controlling mechanism of normally pigmented cells was studied to furnish a further basis for comparison. As a result of these studies the following hypothesis is presented to account for the formation of pigment cells about the cyst. It is known that melanin is produced as the result of a series of complex chemical reactions involving for example, an enzyme such as tyrosinase and the chromogen, tyrosine. It is suggested that certain cells of fish contain such a necessary enzyme but lack the chromogen. The parasite at the time of penetration (or shortly afterwards) supplies the necessary substance for pigment production thus accounting for the characteristically melanated cysts. The dearth of pigment bearing cells about some cysts (as *Clinostomum*) may be due to the absence of the necessary chromogen in the parasite. Further evidence is being sought on these points.

72. *Observations on the Geographical Distribution of Digenetic Trematodes of Marine Fishes.* H. W. MANTER, University of Nebraska.

Comparisons are made of trematodes of the author's collections from Tortugas, Florida (about 175 species), and from the tropical American Pacific (82 species) and of trematodes reported from Bermuda (about 26 species) and from Woods Hole (about 70 species). Assuming that the less known regions have been so sampled as to furnish a representative segment for comparison with the Tortugas region, a few pronounced similarities and dissimilarities among these regions are suggested. In spite of incompleteness of the data it seems safe to conclude the following. (1) Bermuda trematodes are exceedingly similar to those of Tortugas and rather distinct from trematodes at Woods Hole. (2) Trematodes of the tropical American Pacific have a pronounced similarity to the Tortugas fauna (over 28 per

cent identical). (3) Woods Hole trematodes show pronounced Arctic and also Beaufort, North Carolina, connections and about 12 per cent identity with Tortugas species. (4) The distribution picture can be largely explained by oceanic currents and temperatures, past and present. (5) Because of their abundance and variety the digenetic trematodes constitute a favorable group for distribution studies.

73. *The Frequency of Helminths in Their Naturally Infected Hosts.* H. F. HSÜ AND S. Y. LI, Peiping Union Medical College, Peiping, China.  
No abstract received.

74. *Further Studies on the North American Hirudinea.* MARVIN C. MEYER, New Jersey College for Women of Rutgers University.

Our knowledge of the North American fresh-water Hirudinea fauna is so meager that any definite distribution and host record is of considerable importance. Various collections, especially Piscicolidae, received for identification have yielded specimens from remote localities and new hosts. It has been shown that certain species of fresh-water fish leeches may occur in epidemic proportions and may even appreciably affect the yield of economically important fish. Consideration will be given to host-parasite specificity among the Piscicolidae. Several instances of a glossiphoniid infecting the buccal cavity of waterfowl together with its effect will also be discussed.

75. *A New Species of Branchiobdellid from Kentucky.* CLARENCE J. GOODNIGHT, Brooklyn College.

In the examination of a collection of branchiobdellids from near Lexington, Kentucky, a new species was encountered. This new worm was a member of the common eastern genus *Cambarincola* Ellis. It is apparently a form of local distribution, since it was not encountered in collections from other localities in Kentucky. This worm lived on the crayfish, *Cambarus bartoni*. This record gives further evidence to the theory that while most species of branchiobdellids are widely distributed, a few forms are quite local.

76. *Life History and Development of Macracanthorhynchus ingens and Mediorhynchus grandis (Acanthocephala).* DONALD V. MOORE, Rice Institute.

Eggs of *Macracanthorhynchus ingens* from the raccoon, *Procyon lotor*, were fed to grubs of *Phyllophaga crinita*. Hatching in the gut of the grub the acanthor penetrates into the hemocoel where after 60-70 days the encysted acanthella may be found. The acanthor is covered with cuticular spines, and has 3 pairs of rostellar hooks. Thirty to 35 days after infection the larvae may be found attached to the outer wall of the gut. By this time the anlagen of various adult structures may be identified in the central nuclear mass. The elongate pre-acanthella stage has a ring of 6 nuclei in the apical ring of the proboscis, and 14 nuclei in the lemniscal ring, of which 7 migrate into each lemniscus. The acanthella with the proboscis invaginated, which is characteristic of the infective stage, is enveloped by a hyaline fusiform cyst, one cell layer thick, which develops from the outermost layer of the larva.

Eggs of *Mediorhynchus grandis* from the meadowlark, *Sturnella magna*, were found to develop in grasshoppers, *Chortophaga viridifasciata australior*, *Orphuella pelidna* and *Arphia luteola*. The acanthor were found on the exterior of the gut wall 10-12 days after infection and the infective larvae developed in the hemocoel of the grasshoppers in 25-30 days. The acanthor is spined and has 3 pairs of rostellar hooks. There are 6 nuclei in the apical ring of the proboscis and 12 nuclei in the lemniscal ring. The acanthella with the proboscis invaginated, is enveloped by a cyst similar in structure to the cyst of *Macracanthorhynchus ingens*, but is ellipsoid in shape.

77. *An Addition to the Life History of Leidynema appendiculatum (Leidy, 1850)* Chitwood, 1932, a Nematode Parasitic in Cockroaches. A. C. TODD, University of Nebraska.

Observations on the development of the egg of *Leidyneia appendiculatum* have been made during the course of some hatching experiments. These experiments show that two molts occur within the egg during the development of the larva. Under natural conditions the first molt occurs outside the host and results in the establishment of a resting stage which is infective for the roach hosts. The second molt occurs, under natural conditions, inside the roach; it can be demonstrated in vitro by treating infective eggs with dilute protein solutions.

78. *Removal of Chicken Tapeworms by Host Starvation and Some Effects of Such Treatment on Tapeworm Metabolism.* W. M. REID, Monmouth College, AND J. E. ACKERT, Kansas State College.

The loss of strobilae of *Raillietina cesticillus* after 24 to 48 hours of host starvation (Reid, 1940, J. Parasitol. 26 Suppl: 16) suggested the use of starvation in tapeworm control. However, the fact that some scolices were retained in the gut for as much as three weeks of starvation, new strobilae being subsequently regenerated, makes such a procedure impractical. This starvation effect is probably the essential factor in various "treatments" suggested to poultry producers. A claim that treatment may be effected by limiting the diet to oats for a few days appears to be based entirely on concomitant starvation effects. Under experimental conditions substitution of oats for the normal ration resulted in partial or complete voluntary host starvation for one or two days together with the elimination of strobilae. Later, when the oats were taken in quantity, the worms were again able to develop gravid proglottids. Further studies on the chemical composition of worms removed from the gut of feeding fowls as compared with worms from fowls which had been off feed for 20 hours showed no significant change in fat content but a relative rise in nitrogen and water. These findings substantiate the view that the loss of strobilae in starvation is due primarily to depletion in the glycogen reserve to such an extent that muscular energy is no longer available for the worms to maintain their positions.

79. *Effect of Atabrine upon Experimental Cysticercosis of Mice.* JAMES T. CULBERTSON AND SYLVIA H. GREENFIELD, Columbia University.

Thirty-two mice were fed 500 onchospheres of *Taenia taeniaeformis*. Twenty-two of the animals were treated with 5 mg of atabrine for two days before infection, and thereafter on alternate days during the ensuing four weeks. The treated animals and the controls were then autopsied and the cysticerci on the surfaces of their livers were counted. At autopsy, all the control mice were heavily infected, an average of 119.9 living cysts and 9.2 dead cysts being counted. Among the 22 treated animals, an average of 12.7 living cysts and 23.7 dead cysts were found. No living cysts whatsoever were found in 11 of the treated mice. It thus appears that the development of *Cysticercus fasciolaris* in mice is prevented or retarded by the administration of large doses of atabrine.

80. *The Life Cycle of Dioctophyma renale, the Giant Kidney Worm of Mammals.* ARTHUR E. WOODHEAD, University of Michigan.

Eggs of *D. renale* will hatch in the intestine of branchiobdellids. Movement begins in one minute, hatching is finished in five minutes, and penetration into the body cavity is finished in 15 minutes. At about the 10th day the anterior third of the larva swells and the three pharynx lobes, with chitinous elongations, begin to form. Cephalic papillae are plainly seen. Later the larva begins to coil the posterior two-thirds and the body cells increasing in number hide internal development. At six weeks the coiled larva is forming a tightly coiled cyst. Its great similarity to the cyst of *Paragordius* is startling. It is evident that the life cycle of *D. renale* has been concealed by this close similarity. Only in the manner of coiling of the posterior third, can the two cysts be distinguished.

Numerous cysts of *Paragordius* are found in branchiobdellids and we have also found a larva of *D. renale* in the body cavity. The number of *D. renale* cysts occurring in nature appears to be small. Crickets which serve as second intermediate hosts for *Paragordius* are found also to contain a larval form with elongated body

and a retracted head organ of the *D. renale-Paragordius* type. In specimens up to 20 mm there appears to be no degeneration of the head structures. This size larva and its head organs would suit conditions for penetration required of a *D. renale* larva. Feeding experiments are in progress to determine if this form is the larva of *D. renale*.

81. *The Possibility of Chemical Control of the Snail Intermediate Hosts of Schistosoma mansoni in Venezuela.* GEORGE W. LUTTERMOSER. Instituto Nacional de Higiene, Caracas, Venezuela.

A comparison was made of the efficacy of copper carbonate and solutions of recently slaked lime for killing the common snail intermediate hosts of *Schistosoma mansoni* in Venezuela. A 1:1,000 solution of lime or a 1:10,000 solution of copper carbonate killed all these snails in 48 hours. In the presence of soil, a greater concentration of both chemicals was necessary, i.e., at least 190 grams of lime per square meter of water area or about a 3:1,000 solution. The use of the lime is recommended, because in solution it also killed the miracidia and cercariae of *S. mansoni*; it is strongly bactericidal; and it proved nontoxic to rats, mice, a dog, a monkey and a calf. Furthermore, lime is readily available, is much cheaper and much easier to use and may help to neutralize the soil which is acid along many irrigation canals in Venezuela.

Of five typical areas selected for field-test, three were parts of irrigation canals, and the other two were water reservoirs. Movement of snails into or out of these areas was prevented by screen doors. Two days before and after the lime treatment in each region, collections of 200 or more snails were made and if they did not respond within 48 hours to favorable conditions or to different stimuli, they were called "dead." Some of each collection were dissected. Before any treatment, 93, 100, 100, 97 and 55 per cent were alive. After two applications of lime, about 14 days apart, the snails were all killed in two of the five areas, while in the other three areas the percentage of living snails was reduced to 2, 1, 2, 5, and 2 per cent.

82. *Effects of Duodenal Mucus of Dogs and Swine upon the Viability of Ascaridia lineata in Vitro.* L. L. EISENBRANDT, University of Kansas City, AND J. E. ACKERT, Kansas State College.

Ackert, Edgar and Frick (1939) showed that a saline extract of the chicken intestinal mucosa did not kill the chicken nematode, *Ascaridia lineata*, when cultured in vitro. Similar studies were made upon the viability of 349 nematodes of the same species using duodenal mucus extract of dog and of hog. Control worms were placed in a nutrient salt solution. The mortality of *A. lineata* cultured in dog mucus extract was: 24 hours, 35 per cent; 48 hours, 96 per cent and 72 hours, 100 per cent. The death rate of control worms for the same respective periods was: 3 per cent, 8 per cent and 17 per cent. Essentially the same results were obtained with hog duodenal mucus extract; the mortality at 24, 48 and 72 hours was: 0 per cent, 81 per cent and 100 per cent. During the same periods control worms died as follows: 0 per cent, 0 per cent and 28 per cent. Some of these worms lived for a week in the nutrient medium. The difference between the mortality rate of the experimental worms and the control worms in both groups for each period was statistically significant. Therefore, mucus extracts of both dog and hog caused the early death of all the fowl nematodes tested. This may suggest that the host specificity of *Ascaridia lineata* is attributed, in part, to duodenal mucus.

83. *The Role of Duodenal Mucus in Age Resistance.* L. P. FRICK AND J. E. ACKERT, Kansas State College.

On the basis of the close relationship exhibited between the increase in the number of goblet cells in the intestine of older chickens and the development of an age resistance in chickens to the fowl nematode, *Ascaridia galli*, there was reason to believe that some substance might be secreted with the mucus from the duodenal goblet cells which might be instrumental in the development of the age resistance.



Data obtained from tests in which *A. galli* were cultured in vitro in the presence of duodenal mucus from resistant chickens indicated that this mucus had the ability to inhibit the growth of the worms. Furthermore, it was shown that the active agent in the mucus was thermostable. These tests were repeated using numbers of samples large enough for statistical treatment. *A. galli* from 30 to 90 days of age taken by 10-day intervals were treated with mucus from chickens 60 to 140 days of age considered by 20-day intervals. The mucus used in these tests was not treated with heat before use.

The data in this series of experiments when analyzed by analysis of variance yielded the highly significant F value of 6.35. It has thus been demonstrated that the duodenal mucus samples differed significantly in their ability to inhibit the growth of the nematodes, and that the effectiveness of the mucus as a growth inhibitor varied directly with the age of the chickens from which the mucus was taken.

84. *Intestinal Resistance to a Second Infection of Trichinella spiralis*. ARTHUR J. LEVIN AND TITUS C. EVANS, University of Iowa.

As previously reported (Levin and Evans, 1940, J. Parasitol. 26 Suppl: 31), it was possible to treat *Trichinella spiralis* larvae with roentgen radiation (3250-3750r) which inhibited the development of offspring without preventing the maturation of the larvae in the intestine of the host. In this way, irradiated larvae established an intestinal infection in rats without the usual consequent muscle phase. The next step was to determine whether an intestinal infection of *T. spiralis*, without the resulting muscle invasion, would grant host resistance to subsequent *Trichinella* infections. Therefore, 3 groups of rats were infected with irradiated (3250r, 3500r, and 3750r) larvae. The first group was re-infected with normal larvae after two weeks, the second group after 3 weeks, and the third after 4 weeks. Three rats, of each group, were re-infected with a dose of 2,500 larvae and three were re-infected with a heavier dose (10,000 larvae). Two months after the last rat was re-infected, all of the rats were killed and larval counts were made. The control rats (untreated) gave larvae-larvae ratios of 42.81 (heavy infection) and 66.00 (lighter infection). The 18 treated rats gave an average ratio of 0.49, with one extreme case of 12.50. The treated rats were as resistant to heavy re-infection doses as they were to lighter ones.

85. *Demonstration of Naturally Acquired, Passive Immunity to Hymenolepis nana var. fraterna*. JOHN E. LARSH, JR., Johns Hopkins University.

Passive transfer of immunity from mother mice to their offspring has been demonstrated for the cestode, *Hymenolepis nana* var. *fraterna*. At the time of weaning (around 21 days), mice born of an infected mother showed an average of about 0.7 per cent development of cysticercoids from a test infection, in contrast to an average of at least 6 per cent in the young of uninfected mothers. This resistance to infection persists for about 38 to 40 days. The immunity is passively transferred from mother to offspring both in utero and in the milk. That received in utero is ephemeral and of slight significance compared with that obtained from the milk. Infected and uninfected mothers were exchanged immediately after the young were born, so that an infected mouse would raise the litter born of an uninfected mouse, and vice versa. When these young were infected 21 days after birth, those which were protected by the milk alone showed a percentage development of cysticercoids of about 0.7 per cent; those which received protection only in utero about 1.6 per cent; and the controls about 6 per cent. When mice were infected after parturition, it was found that the offspring were as well protected as those born of mothers which had been infected early in pregnancy. Mice that had lost their infection more than a month before conception were still capable of transmitting protective antibodies to their offspring. This demonstration of passive transfer of immunity from mother to offspring lends support to the evidence already available that an antibody is involved in immunity produced against this parasite.

86. *Effects of Testosterone on Nematodes*. LYELL J. THOMAS, University of Illinois.

Nematodes of the genus *Rhabditis* were obtained from the body cavity of the carrion beetle *Necrophorus obicolis* at the University of Michigan Biological Station, August 18, 1940. These nematodes were cultured successfully and on April 25, 1941, a few crystals of testosterone were added to a culture (strain A). Worms were transferred from it to fresh culture media without testosterone, one month apart, in July, August and September, 1941, but all males examined showed the spicules to be fused from the distal end part way so as to form a V-shaped structure. Control cultures showed the males to have normally two equal spicules. On October 9, 1941, additional crystals of testosterone were given to the fused-spicule strain, and when examined October 20, 1941, all males examined had but a single large spicule. The females were overly large and either sterile or their egg production greatly reduced. This reaction to testosterone on the part of female nematodes is similar to that in the female mammal.

87. *Hairworms as Parasites of Fishes*. ROSS F. NIGRELLI, New York Aquarium.

From April to July of the present year, several tropical, semi-tropical and temperate fresh-water fishes present in the exhibition tanks of the New York Aquarium were found infected with hairworms (Gordiaceae). The small tropical and semi-tropical hosts, *Limia dominicensis* C. & V., *Poecilia vivipara* B. & S., *Poecilibrycon* sp., and *Platypoecilus maculatus* Günther, had bellies so distended that they found it difficult to swim or keep themselves right side up. On rupturing the belly wall a serous exudate was released. In all cases two active worms, male and female, varying in length from 65 mm to 215 mm, were found free in the body cavity and strongly coiled around each other. Further examination of the fishes showed that the organs were displaced and considerably damaged. The temperate fresh-water fishes, *Salmo irideus* Gibbons, *Salvelinus fontinalis* (Mitchill), and *Micropterus punctulatus* (Raf.), showed no belly enlargement, and, with the exception of *M. punctulatus* (Kentucky bass), two worms, male and female, measuring 125 mm and 135 mm respectively, had penetrated the sinus venosus resulting in a severe hemorrhage. All the worms were allocated to the genus *Chorodes* Creplin, 1847. Those found in the Kentucky bass and the trouts were definitely identified as *Chorodes morgani* Montgomery, 1898. Both males and females showed the characteristic distinction of the cuticular areoles and the caudal ends.

88. *Cultivation Experiments with the Avian Malaria*, *Plasmodium circumflexum*. FREDERICK COULSTON, Syracuse University.

A cultivation method was sought that would keep the erythrocytes suitable for growth of the parasite, supply oxygen and carbon dioxide and permit agitation of the culture mixtures. Many types of containers were used. The best results were obtained with round-bottomed test tubes. These were bent almost at right angles, 3.5 cm from the bottom, and indented below the bend. Thus the end of the tube became a separate compartment communicating by a narrow opening with the upper portion. This shape helped preclude contamination and prevented the mixture from running up the sides of the tube. The agitator was mounted in an incubator (35–39° C). Agitation was controlled by a time clock. Good results were obtained with the following mixture: 1 part each of 1:1000 heparin, normal blood, heavily parasitized blood, and 2 parts Baker's tissue culture fluid. Baker's fluid is a buffered solution containing glucose with many of the essential proteins and vitamins. Sterile oxygen and carbon dioxide (5 per cent) were added by needle. Fresh erythrocytes were supplied daily.

*P. circumflexum* was grown for at least one asexual generation. In some experiments, of two weeks duration, possibly three and four generations developed. There was usually a definite increase in the number of parasites from the third to the seventh day. Good cultures, 1 to 13 days old, produced infections in canaries. Invasion of freshly supplied erythrocytes was easily demonstrated. After three days most large schizonts and segmenters were observed growing in remains of erythrocytes. Abundant pigment appeared associated with the characteristic vacuole. Occasionally the pigment was very refractile resembling *P. vaughani*. Gametocytes could be recognized for 7 days.

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# ABSTRACTS

41

## *List of Meeting Places*

1925	Kansas City	1930	Cleveland	1936	Atlantic City
1926	Philadelphia	1931	New Orleans	1937	Indianapolis
1927	Nashville	1932	Atlantic City	1938	Richmond
1928	New York	1933	Boston	1939	Columbus
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OF PARASITOLOGISTS

(Including revisions of Dec. 30, 1931, Dec. 28, 1932, Dec. 29, 1933, and Dec. 31, 1940)

## NAME AND OBJECT

The name of the society is the American Society of Parasitologists.

The object of the society is the association of workers in the field of Parasitology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as will tend to the advancement of parasitological teaching and investigation in this country.

## MEMBERSHIP

The members of the society shall be of two classes, active and foreign honorary.

Any person interested in parasitology may be a candidate for active membership.

Any foreign scientist who has made eminent contribution to Parasitology may be eligible for honorary membership.

Candidates for membership shall be elected by the Council.

## OFFICERS

The officers of the society shall be a President and a Vice-President, who shall be elected for one year; a Secretary and a Treasurer, who shall be elected for two years; and members at large of the Council.

The Council shall consist of the President, the Vice-President, the Secretary, the Treasurer, and eight members elected by ballot from the society at large, two for four years, two for three years, two for two years and two for one year. After the first year, two members at large of the Council shall be elected each year to serve four years.

If any vacancy occurs among the officers, the Council is authorized to appoint a member to fill out the unexpired portion of the current year.

The routine business of the society shall be administered by the Council.

Five shall constitute a quorum of the Council.

## DUES

The dues, to include subscription to the JOURNAL OF PARASITOLOGY, shall be four dollars the year for active members, unless changed by vote of the society.

## MEETINGS

There shall be an annual meeting and such other scientific or business meetings as the Council shall determine.

During the annual meeting a business meeting will be held for the election of officers for the ensuing year and for the transaction of other business.

## JOURNAL OF PARASITOLOGY

This Journal, the property of the society, is its official organ. Responsibility for its conduct shall rest with the Council which shall select the editorial staff and set the price of subscription.

## ENDOWMENT FUND

Provision is made for the establishment of a permanent endowment fund, the principal of which may be expended only by a three-fourths vote of all members of the Council and approval by a three-fourths vote of the members of the society present at a regular meeting. The Council shall be entrusted with the maintenance of the fund, and the use of the income therefrom.

## AMENDMENT

On recommendation of a two-thirds vote of the Council, the constitution may be amended by a two-thirds vote of the members present at any regular business meeting of the society, provided that at least 30 days' notice has been given to the membership of the proposed amendment.

## BY-LAWS

## MEMBERSHIP

1. *Election of Members.* An affirmative vote of all Council members present at a meeting shall be necessary for the election of candidates for membership. If the vote is taken by mail ballot, an affirmative vote of all members of the Council replying within thirty days shall be required.

2. *Members in Good Standing.* Members in good standing are those whose current dues are paid.

3. *Delinquent Members.* The JOURNAL OF PARASITOLOGY shall not be sent to members in arrears, and members in arrears for the current year and two previous years shall be dropped from the roll of the society at the end of the current year.

4. *Reinstatement of Delinquent Members.* Members dropped for non-payment of dues, or who have resigned, may be reinstated by the payment of all dues in arrears. Otherwise, the applicant must apply for election as a new member.

5. *Non-subscribing Members.* Where a second membership in the society is taken in the same immediate family, the second member may join upon the payment of annual dues of one dollar, but without receiving the JOURNAL OF PARASITOLOGY.

6. *Honorary Life Members.* Upon unanimous vote, the Council may recommend that the society confer honorary life membership upon distinguished American parasitologists over sixty years of age. Honorary Life Members shall enjoy full membership privileges and shall be exempted from the payment of dues. The number of Honorary Life Members at any one time shall be limited to five.

7. *Foreign Honorary Members.* Upon unanimous vote, the Council at its annual meeting may elect Foreign Honorary Members. No two men from the same country shall be elected in the same year. The number of Foreign Honorary Members at any one time shall be limited to twelve. Foreign Honorary Members may receive the JOURNAL OF PARASITOLOGY upon payment of membership dues.

## PRESENTATION OF PAPERS AT ANNUAL MEETINGS

1. Except for invited papers, and papers coming to the American Society of Parasitologists program through joint sessions with another society or section, all persons presenting papers must be members of the society in good standing, or must be introduced by a member in good standing.

2. Each member of the society in good standing may be allotted not more than fifteen minutes of program time, to be used in person or by a non-member introduced by him. If the program is crowded, the maximal allotment of time may be reduced to ten minutes.

3. Papers offered for presentation too late to be included on the printed program may be presented at the conclusion of any of the scientific sessions provided that the presiding officer obtains the consent of the members present.

## OFFICERS

1. The Council shall act as a nominating committee and at the annual meeting shall submit to the society at least one nominee for each office to be filled. The Secretary shall invite the members of the society to submit nominations for consideration by the Council.

2. The Treasurer of the society shall act also as Treasurer for the JOURNAL OF PARASITOLOGY and shall be bonded for the sum of two thousand dollars.

3. A sum of fifty dollars shall be allotted annually to the Secretary, to the Treasurer, and to the Chairman of the Editorial Committee for attendance at the annual meeting.

## MANAGEMENT OF THE JOURNAL OF PARASITOLOGY

1. The JOURNAL OF PARASITOLOGY, as the official organ of the society, shall be managed by an Editorial Committee appointed by the Council for a five-year period, and shall consist of one Protozoologist, one Helminthologist, and one Entomologist, one of whom shall be appointed by the Council to act as Chairman.

2. The Editorial Committee shall be assisted by an Editorial Board consisting of twelve members appointed by the Council for a four-year period in such a way that three members will retire and three new members shall be elected each year. The Editorial Board shall consist of two Entomologists, four Protozoologists, and six Helminthologists to be elected on the basis of attainment, interest in the society and geographical location.

3. The price of the JOURNAL OF PARASITOLOGY shall be five dollars per volume, except to members of the American Society of Parasitologists who shall receive it as a membership privilege included in the annual dues of four dollars.

## ENDOWMENT FUND

1. Council shall select a Custodian of the Endowment Fund and two associates to whom it may delegate responsibility for management of the fund. The Custodian shall make an annual accounting to Council and such other reports as Council may request. The approval of two of the three custodians shall be necessary for the purchase, sale or exchange of securities. One of the three custodians shall be the Treasurer of the society and his signature shall be required on all vouchers of expenditure from the fund.

## ADDITIONS AND AMENDMENTS

1. Additional by-laws may be created by a two-thirds vote of Council members present at a meeting, or by an affirmative vote of nine Council members in a ballot conducted by mail. By the same procedure existing by-laws may be repealed, amended, or suspended.

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## THE DIFFERENTIATION OF THE EGGS OF THE TRICHO- STRONGYLID SPECIES *NEMATODIRUS* *FILICOLLIS* AND *N. SPATHIGER*

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In his description of species of *Nematodirus* May (1921) was unable to distinguish clearly between the females of species; two of these were *N. filicollis* and *N. spathiger*. Among characters which he considered, were dimensions of eggs, and these were found to overlap between species. Thus he records for the eggs of *N. filicollis* a range of 130–200  $\mu$  in length by 70–90  $\mu$  in width and for *N. spathiger*, respectively 150–220  $\mu$  and 80–110  $\mu$ . Tetley (1935) pointed out that if attention were paid to dimensions embracing the greater number of eggs of these species, specific differences could be observed, for the majority of the eggs of *N. filicollis* were grouped within the limits of 140–165  $\mu$  by 70–85  $\mu$  and those of *N. spathiger* within 180–210  $\mu$  by 90–105  $\mu$ . Shorb (1940) found that the dimensions for *N. filicollis* ranged from 147.2–195.7  $\mu$  by 72.8–108.3  $\mu$ . From the measurements he has plotted, though small in number, it is apparent that the majority fell nearer the lower limits of this range.

A female of *Nematodirus* has been found in which the eggs of each uterus, respectively, fell centrally in the *N. filicollis* and *N. spathiger* groupings. While this is regarded as exceptional, it is considered that in view of the possible use of egg measurements in specific identification of females of this genus, the evidence providing the basis for previous conclusions, together with more recent findings, should be made known.

### MATERIALS AND METHODS

Material for measurement was obtained mainly from the dissection of females; the data pertaining to fecal eggs were collected in the course of applying the Stoll dilution egg counting method. Eggs dissected from females were measured as soon as possible after removal of worms from

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the sheep at autopsy, this sometimes involving a delay of some hours. Fecal eggs, as a rule, were exposed to the action of N/10 NaOH for several hours. Some material was fixed in hot 5 per cent formalin within a few moments of the death of the host. The specific numbers of males of *N. filicollis* and *N. spathiger* were taken as indices of the numbers of females of the two species. Measurements were made using an ocular micrometer.

## OBSERVATIONS

*Sheep I.*

Breed: Romney cross.

Age: approximately three months.

Slaughtering date: December 7, 1932.

Place of origin: Palmerston North, New Zealand.

Two hundred males of *Nematodirus* were examined; these were found to consist of 199 *N. filicollis* and 1 *N. spathiger*. Measurements were made of 101 eggs dissected from 7 worms selected at random. The results are plotted in Fig. 1.

*Sheep II.*

Breed: Romney cross.

Age: four months.

Slaughtering date: May 21, 1936.

Place of origin: Palmerston North, New Zealand.

This animal was slaughtered in an advanced state of exhaustion, the outcome of parasitic gastritis. Material was fixed in hot 5 per cent formalin. The infection of *Nematodirus* was drawing to a close, as shown by the scattering of parasites throughout the digestive tract from the duodenum to the rectum. Males of *N. filicollis* and *N. spathiger* were present in approximately similar numbers. From each of 62 females taken at random at least one representative egg was extracted. These and eggs found free in the lumen of the digestive tract, were measured, 119 of which measurements are plotted in Fig. 2. Abnormal eggs found in this material have been omitted from Fig. 2 and are discussed in another part of the paper.

*Sheep III.*

Breed: not known.

Age: a few months old.

Slaughtering date: November 15, 1939.

Place of origin: Topeka, Kansas.

Examination of 40 males of *Nematodirus* resulted in finding 37 of *N. spathiger* and 3 of *N. abnormalis*. Forty females taken at random were found on cursory examination to contain eggs of uniform shape



and size; of these, 20, again selected at random, were dissected and one typical looking egg from each measured. The results are plotted in Fig. 3.

*Sheep IV.*

Breed: Southdown Romney cross.

Age: approximately twelve months.

Dates of fecal examination: during 1935.

Place of origin: Palmerston North, New Zealand.

The data were derived from eggs encountered while making dilution fecal counts by the Stoll technique. These are plotted in Fig. 4. Post-mortem examination was not made, but from autopsies on other animals reared in the same paddock it was found that *N. filicollis* and *N. spathiger* were abundant while *N. abnormalis* and *N. helvetianus* were rarely present and then only as odd individuals.

*Aggregated Results*

In addition to the foregoing data, the series was enlarged through the study of eggs from other worms, obtained from Romney cross lambs located at Palmerston North. In Fig. 5, the measurements of 280 of these are graphed, together with the 279 already shown from sheep 1-4, as a total sample of 559.

To indicate something of the range of experience contained in the results, the dates and places of origin, number of sheep providing material, and the numbers of worms dissected are set out in Table 1.

TABLE 1. *Sources of material*

Origin		Number of sheep providing material	Source of eggs : Number of worms
Date	Place		
1932 Nov.	Palmerston N., N. Z.	1	1
" Dec.	" " "	1	1 (See Fig. 1)
1934 Nov.	" " "	2	2
" Dec.	" " "	3	3
1936 May	" " "	1	62 (See Fig. 2)
1938 Jan.	" " "	1	3
" Feb.	" " "	3	19
1939 Nov.	Topeka, Kans.	1	20 (See Fig. 3)
1935 }	Palmerston N., N. Z.	1	Feces (See Fig. 4)
1936 }			
Total .....		14	111 (at least)

*Irregularities in Dimension of Eggs*

Occasionally when examining fecal material abnormally small *Nematodirus* eggs were found and, once, an infection (sheep II) was found in which females contained eggs of irregular dimensions. Among 62 females from this latter source 8 contained eggs which departed in one or more respects from the normal. One worm was found to contain eggs of differing dimensions between the two uteri. Thus, of the 7 uni-

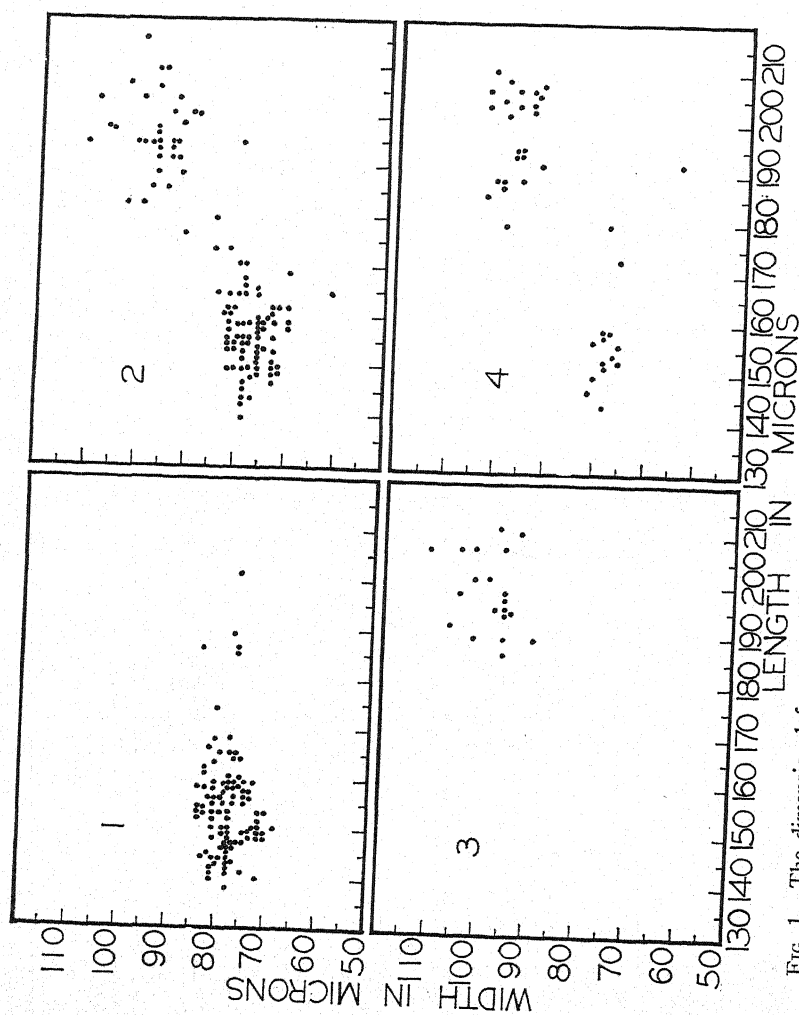


FIG. 1. The dimensional frequency distribution of eggs dissected from worms from sheep I in which *N. filicollis* males predominated in the ratio of 199:1 over those of *N. spathiger*.

FIG. 2. The dimensional frequency of eggs dissected from worms from sheep II in which males of *N. spathiger* and *N. filicollis* were in approximately similar numbers.

FIG. 3. The dimensional frequency distribution of eggs dissected from worms from sheep III in which males of *N. spathiger* were present and those of *N. filicollis* absent.

FIG. 4. The dimensional frequency distribution of fecal eggs from sheep IV. See text.

form eggs of the anterior uterus, there were 6 with the dimensions:  $195 \times 85 \mu$ ,  $195 \times 93 \mu$ ,  $198 \times 93 \mu$ ,  $192 \times 96 \mu$ ,  $192 \times 99 \mu$ ,  $198 \times 90 \mu$ ; and among the equally uniform eggs of the posterior uterus 6 were of the following sizes:  $171 \times 78 \mu$ ,  $168 \times 78 \mu$ ,  $160 \times 81 \mu$ ,  $171 \times 81 \mu$ ,  $168 \times 81 \mu$ ,  $180 \times 81 \mu$ .

Each of the remaining 7 worms, exhibiting irregularities, contained one or more abnormal eggs sandwiched between seemingly normal eggs.

It was apparent, from the degree of segmentation and number of eggs, that egg production by the 8 females was below that of other members of the population among which, in turn, the rate fell below the normal for the species. Malformed eggs were not to be found among the 1,024 eggs found free in the contents of the alimentary canal of sheep II.

#### DISCUSSION

Eggs of *Nematodirus* species, partly on account of their thick resistant shells, are not readily subject to change in shape and volume through fluctuations in osmotic or mechanical pressure. The similarity of the results for dissected out eggs (Figs. 1, 2 and 3) to those for fecal eggs (Fig. 4) was therefore not unexpected.

It is seen from Figs. 1, 2, 3, 4, and 5 that two distinct groupings of eggs were present; the obvious conclusion is that two species were con-

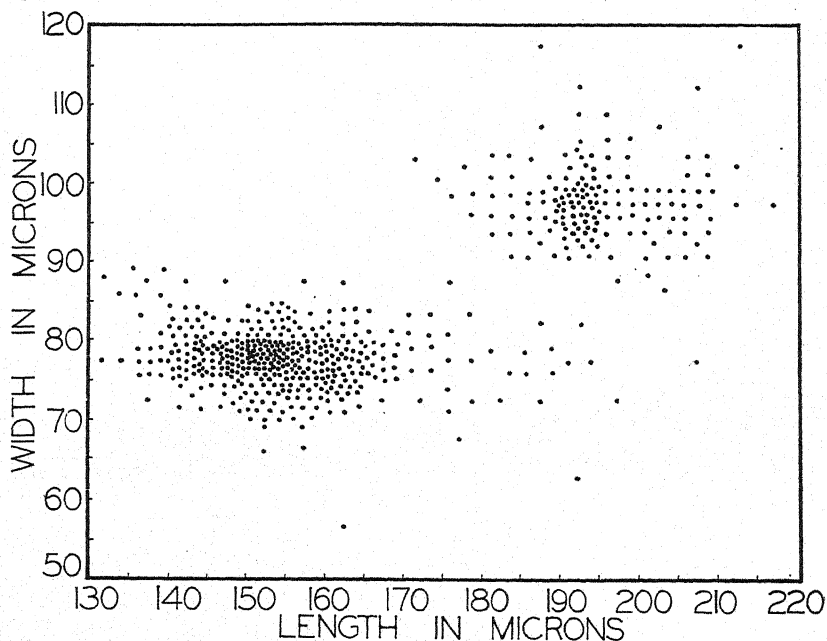


FIG. 5. The dimensional frequency distribution of *Nematodirus* eggs from all sources, including those in Figs. 1-4, supplemented by 280 others. It will be seen that the identity of the specific groupings is distinct.

cerned. Males of species other than *N. filicollis* and *N. spathiger* were so rarely found that the other species will be disregarded as insignificant in the present discussion. There can be no doubt therefore that the groupings were of *N. filicollis* and *N. spathiger*.

In sheep I the finding of males in the proportion of 199 of *N. filicollis* to 1 *N. spathiger* indicated the probability that females taken for dissection were of *N. filicollis*. The data from this sheep (Fig. 1) reveal that there was a dimensional range among the eggs of 138–201  $\mu$  by 69–84  $\mu$ .

The even division of the males of the two species, *N. filicollis* and *N. spathiger*, observed by inspection in sheep II, is reflected in the two groupings of the eggs dissected from this animal (Fig. 2). One grouping, 140–170  $\mu$  by 61–85  $\mu$ , coincided with that found in sheep I and it is concluded was of *N. filicollis*. That the second grouping, 180–215  $\mu$  by 85–110  $\mu$ , was *N. spathiger* is clinched by the results from sheep III (Fig. 3). A sample population from this animal contained 37 males of *N. spathiger* to 3 of *N. abnormalis*, and this, it is believed, indicated that *N. filicollis* was absent. The range of egg measurements, therefore, found in this animal, of 185–210  $\mu$  by 90–110  $\mu$  finally established that the eggs of the two species under discussion were distinct in size.

The results from sheep IV (Fig. 4) show that the groupings were distinct among eggs of fecal origin; and the aggregated results (Fig. 5) indicate that despite the miscellaneous origin of the data the groupings retained their identity. It is therefore concluded that the differences found were of general occurrence.

In view of the centering tendency found in the data, it was considered that a statistical treatment in which attention was paid to range of distribution in conjunction with intensity of population would enable inter-specific differences to be more clearly demonstrated.

The specific population groups were regarded as resembling a bivariate normal frequency distribution sufficiently closely to permit the application of formulae relevant to the latter. Thus the range of the mean plus and minus twice the standard deviation which, in a universe, would enclose 91.2 per cent of the population, was estimated for the data from sheep I and the aggregated data for the arbitrarily defined specific populations. These ranges, to the nearest micron, are given in Table 2. It can be seen, for *N. filicollis*, that the range for sheep I closely approximated that for the aggregated results. This would indicate that only a small degree of inter-host fluctuation in the size of eggs of this species took place. While data, equivalent in every respect, were not available for *N. spathiger*, there was no reason for suspecting that a different state of affairs obtained for this species. It was considered, therefore, that statistical comparison of the populations of *N. filicollis* and *N. spathiger* could be reasonably made.

TABLE 2.—The mean axial lengths of eggs of *Nematodirus filicollis* and *N. spathiger* and the computed range of distribution of 91.2 per cent of their populations

Species	Means		Distribution of 91.2% of eggsc	
	Length $\mu$	Width $\mu$	Length $\mu$	Width $\mu$
<i>N. filicollis</i> <sup>a</sup> .....	154.55 $\pm$ 0.71	77.47 $\pm$ 0.23	133–176	71– 84
<i>N. filicollis</i> <sup>b</sup> .....	153.92 $\pm$ 0.34	77.16 $\pm$ 0.14	134–174	69– 86
<i>N. spathiger</i> <sup>b</sup> .....	194.64 $\pm$ 0.44	97.27 $\pm$ 0.44	179–210	88–107

<sup>a</sup> From sheep I.<sup>b</sup> From aggregated results (see Fig. 5).<sup>c</sup> The statistical range of the mean plus and minus twice the standard deviation.

The ranges of distribution of 91.2 per cent of the populations of *N. filicollis* and *N. spathiger* are seen in Table 2 not to have overlapped. In that these ranges were separated and that only a fraction of the populations of the outer statistical zone (containing 8.8 per cent of populations) overlay one another, the individual chances of eggs of one species falling within the 91.2 per cent zone of the other, were small. The partial overlapping of the outer zones of quadrants of the distributions reduced individual chances of falling within the extreme range of the second species, to within the vicinity of one in forty; the individual chances of falling within the statistical range of 91.2 per cent of the other species were much smaller. It is therefore considered that the range of 91.2 per cent of the populations may be regarded as a specific character so far as *N. filicollis* and *N. spathiger* are concerned.

Possibly in other instances where specific differences between females have not been clearly apparent this method of identification may have application.

It is concluded that the malformed eggs were, from the point of view of the present discussion, of little significance. However, as the host in which these were found was in a state of extreme exhaustion at the time of death and as many of the parasites were in the act of being eliminated, it is possible that they have significance as an expression of a phase of the host-parasite relationship adverse to the parasite species. The finding of even a few malformed eggs would, in this connection, be of importance.

## SUMMARY

By plotting lengths against widths it was possible to distinguish between the eggs of *Nematodirus filicollis* and *N. spathiger*.

The extreme ranges of the respective bivariate normal frequency distributions overlapped but this did not happen within the statistical ranges of means plus and minus twice the standard deviations.

Ranges so computed were, for *N. filicollis*, 133–176  $\mu$  by 69–86  $\mu$  and for *N. spathiger*, 179–210  $\mu$  by 88–107  $\mu$ .

In that these ranges were estimated to enclose 91.2 per cent of the



populations of the two species it is suggested that they have value in the specific identification of females of the two species.

It is further suggested that the method may have wider application in the identification of species where specific differences between females by means of other characters have not been clearly demonstrated.

The finding of even a small number of malformed eggs in the feces of a host, it is concluded, is an index of a pitch of the host-parasite relationship, adverse to the parasite.

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THE EGG-LAYING FUNCTION OF A NEMATODE AS  
SHOWN BY STUDY OF *NEMATODIRUS* EGGS  
IN UTERO

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At present, methods do not exist for measuring exactly the pitch of the host-parasite relationship at one point of time. Vague indications of the potential of the relationship have been obtained by observing (1) the ability of parasites to survive, (2) the size to which parasites have grown, (3) the effects of the parasites on the host over a period of time, (4) the feeding of the parasites, and (5) the fecundity of parasites as shown by fecal egg numbers, these latter being expressed either as an empirical figure or as a mean for the population.

The degree to which the various functions of the parasites are related to those of the host-parasite relationship seems still to be largely unknown. It would be expected that they would be linked. Thus, in connection with egg-laying of many parasites, from the standpoint of quantity of eggs produced, or the total bulk of such eggs, it would appear that egg-laying has a relationship to the feeding of the worms, proceeding either simultaneously or alternately at short intervals, with feeding. To the extent therefore that extraction of food from the host is responsible for the effects, fecundity must be regarded as an index of these effects. Support for this is provided by Stoll and Tseng (1925), who found that the production of eggs by hookworms, indicated by per gram fecal counts, paralleled clinical symptoms of disease caused by these parasites as measured by lowered hemoglobin or blood loss. Then again from the viewpoint of host resistance or the favorability of the host for the parasite, egg production has been used as an index. Stoll (1929), with *Haemonchus contortus*, the same author (1932) and Sarles (1932), with *Trichostrongylus colubratus*, were early in the field to investigate the host-parasite relationship by following the egg production of parasites.

In the belief, therefore, that closer study of egg production by parasites would result in knowledge that might aid in the measurement of the pitch of the host-parasite relationship, an examination has been made of the uterine egg populations of *Nematodirus* species in sheep.

Graham (1938) has investigated the fecundity over a period, of single individuals of *Strongyloides ratti*. The present approach, in essentials, is complementary to this work in that the egg output of whole populations at one point of time has been considered.

*Nematodirus* species were found a convenient experimental medium.

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The eggs of *N. filicollis* and *N. spathiger* are easily distinguishable from one another and from those of other genera; their numbers in utero are relatively small, though sufficiently numerous for effective statistical treatment; counting of cells is particularly easy in the eggs of this genus for segmentation rarely proceeds beyond the 8-cell stage in the host, and up to and including the 16-cell stage, at least, the comparatively large cells, resulting from holoblastic equal division, retain a clear-cut spherical form; eggs are laid in the order in which they are produced by the ovaries. Species were easily procured in abundance.

#### MATERIAL AND METHODS

Material was obtained from Romney cross lambs at Palmerston North, New Zealand.

Following slaughtering of the lambs, material was removed within the space of a few minutes and fixed in hot 5 per cent formalin in order to kill eggs, both within the worms and free in the ingesta.

Examination for the numbers and degree of segmentation of eggs was made on the intact worms, using a dissecting microscope.

Specific identification of females was accomplished from characters of the contained eggs (Tetley, 1935).

#### RESULTS

Data were obtained from five sheep. Numbers I, II, III and IV were spring-born lambs approximately four months of age and in good health at the time of slaughtering; sheep V was a summer-born lamb, also about four months of age at the time of autopsy, but in an exhausted condition as the result of parasitic gastritis. Populations of *N. filicollis* and *N. spathiger* were located, in all five animals, mainly in the anterior part of the small intestine. It was apparent that the infection was in process of being eliminated in sheep V, as parasites were scattered along the whole length of the alimentary canal posterior to the duodenum.

Material was taken from the small intestine of the first four animals without regard to the region of infection. In the instance of sheep V note was made of the section of the alimentary tract from which samples were taken. The accompanying tabulation indicates the number and origin of the female worms providing in utero data.

Sheep	Region of digestive tract	<i>N. filicollis</i>	<i>N. spathiger</i>
I .....	Small intestine	18	6
II .....	" "	15	10
III .....	" "	31	1
IV .....	" "	14	5
V .....	7-12 feet	40	13
	25-30 "	9	30
	31-36 "	5	30
	37-42 "	—	19
	43-48 "	8	36
	Cecum	—	26

Complete data on numbers and degree of segmentation of eggs were collected for the majority of worms above listed. In others the maximum degree of segmentation only was observed.

A condensation of the data is set out as follows:

*Table 1.* The frequency with which worms contained arrays of the various segmentation stages of eggs.

*Table 2.* The frequency with which segmentation stages of eggs were found as maxima for individual uteri.

*Table 3.* The relative frequency of segmentation stages of eggs of *N. filicollis* and *N. spathiger* found free in the lumen of sections of the alimentary canal of sheep V at the time of autopsy.

*Table 4.* The mean frequencies of segmentation stages of eggs in utero.

*Table 5.* The frequency with which one or other uterus predominated in number of unsegmented eggs.

*Table 6.* The frequency of uteri containing unsegmented eggs.

*Table 7.* The frequency of the numbers of unsegmented eggs in individual worms.

*Fig. 1.* The correlation between the anterior and the posterior uteri of individuals of *N. filicollis* in the numbers of unsegmented eggs.

*Fig. 2.* The correlation between the numbers of unsegmented and 2-cell *N. filicollis* eggs in utero.

#### *The Segmentation of Eggs*

On account of the small area of cross section of the lumen of the genital tract, eggs of *Nematodirus* species proceed along its entire length in single file. Thus they come to be laid in the order of their origin in the ovary. Eggs of the anterior uterus may be crowded sometimes, and tend to lose this order; the disruption, however, does not extend as far back as the 2-cell stages. Among the several hundred worms examined, in only one female of *N. filicollis* was the serial order of the eggs lost. This female contained an abnormally large number of eggs in both uteri.

Following entry of eggs to the uterus and fertilization, segmentation begins, the successive cleavages taking place in the order of extrusion of the eggs from the ovary. In this way particular segmentation stages come to be grouped together. Departures from this sequence were rare. Among a total of 108 females of *N. filicollis* and 54 *N. spathiger* from sheep I, II, III, IV and V there were 6 worms of the former species and 2 of the latter in which odd eggs were out of the normal segmentation order. In 3 of the *N. filicollis* females the interruption was associated with obvious size abnormality on the part of the displaced eggs. The remaining instances concerned normal eggs which were located among the next higher segmentation stage. These it is considered were examples of extreme fluctuation that might be expected to occur in the length of time of the various stages.

From the synchronous division of cells in individual eggs and the orderliness of the array of segmentation stages along the uterus it is concluded that the extrusion of successive eggs from the ovaries was at intervals of sufficient length to mask individual fluctuations in time of fertilization and in lengths of the succeeding cleavages.

*The Stage of Segmentation at Which Eggs are Laid and the Development of Eggs in the Alimentary Canal*

The range of the maximum stages of segmentation found in individual worms and the frequencies of the various segmentation stages found in the lumen of the alimentary canal are set out in Tables 2 and 3. From these it is concluded that eggs were being laid by worms at the 2-8-cell stages, in both *N. filicollis* and *N. spathiger* the 3-4-cell stage being the one at which most worms were laying eggs.

The frequently recorded fact that eggs of *Nematodirus* are voided in

TABLE 1.—*The frequency of arrays of segmentation stages in populations of N. filicollis and N. spathiger. An unbroken array has been regarded as one in which all intermediate cleavage stages between unsegmented eggs and the maximum stage found in a uterus, were present*

Species	Sheep	Frequency of array					
		Anterior uterus			Posterior uterus		
		Unbroken	Broken	No eggs	Unbroken	Broken	No eggs
<i>N. filicollis</i>	I	17	1	—	18	—	—
"	II	13	1	—	14	—	—
"	III	30	—	1	31	—	—
"	IV	14	—	—	13	—	1
"	V	25	3	—	19	9	—
<i>N. spathiger</i>	I	6	—	—	6	—	—
"	II	10	—	—	10	—	—
"	III	1	—	—	1	—	—
"	IV	4	—	—	4	—	—
"	V	27	2	1	28	1	1

the feces of the host in the 8-cell stage was confirmed. Occasionally eggs in earlier stages of segmentation were found in feces and once a 16-cell egg was found in material removed at autopsy and fixed with heat within a few moments of the death of the host.

In Table 3 comparison has been made of the percentages for each stage of egg-segmentation of *N. filicollis* and *N. spathiger* from various sections of the digestive tract. The greater part of the populations of these two species was located in the anterior region of the small intestine; therefore the comparison was essentially of eggs that had traversed almost the entire length of the small intestine. It is seen that the percentage of 1-4-cell stages diminished with passage along the intestine, from 68 per cent to 30 per cent in the instance of *N. filicollis* and 61 per cent to 13 per cent in *N. spathiger*. The conclusion is drawn that during the time of this passage many eggs graduated to the 8-cell stage but that no 8-cell



TABLE 2.—The frequency with which segmentation stages of eggs were found as maxima for individual uteri

Sheep	Maximal segmentation class									
	Anterior uterus					Posterior uterus				
	0 <sup>a</sup>	1	2	3-4	5-8	0 <sup>a</sup>	1	2	3-4	5-8
<i>N. filicollis</i>										
I .....	—	2	2	8	6	—	2	8	7	1
II .....	—	3	1	4	8	—	1	3	5	4
III .....	—	—	—	11	18	—	—	3	19	8
IV .....	—	—	1	13	6	1	—	3	10	1
V S.I. 7-12 feet <sup>b</sup>	—	4	6	24	2	1	2	4	11	23
" S.I. 25-30 feet	—	1	—	3	4	2	2	—	1	4
" S.I. 31-36 feet	—	1	—	—	1	2	—	—	—	3
" S.I. 37-42 feet	—	5	—	—	—	2	—	—	—	—
" S.I. 43-48 feet	2	—	—	—	1	2	3	2	—	1
Total .....	7	15	10	63	45	8	10	24	53	45
Percentage .....	5%	11%	7%	45%	32%	6%	7%	17%	38%	32%
<i>N. spathiger</i>										
I .....	—	—	1	1	4	1	1	1	—	3
II .....	—	2	—	—	8	—	—	2	3	3
III .....	—	—	—	—	1	—	—	—	1	3
IV .....	—	—	—	—	5	—	—	—	2	3
V S.I. 7-12 feet	—	—	3	7	3	—	—	3	8	3
" S.I. 25-30 feet	—	—	4	13	10	—	2	6	13	12
" S.I. 31-36 feet	1	1	7	18	4	2	2	8	13	5
" S.I. 37-42 feet	1	1	—	14	4	1	1	5	7	3
" S.I. 43-48 feet	1	1	11	9	14	1	2	10	13	9
" Cecum <sup>c</sup> .....	—	2	4	10	9	—	—	3	—	—
Total .....	4	8	30	72	62	5	9	37	75	50
Percentage .....	2%	5%	17%	41%	35%	3%	5%	2%	43%	28%

<sup>a</sup> 0: number of uteri not containing eggs.<sup>b</sup> S.I. 7-12 feet, etc.: sections of small intestine measured from anterior end.<sup>c</sup> *N. filicollis* not found in cecum.

TABLE 3.—The relative frequency for *N. filicollis* and *N. spathiger* of egg segmentation stages in the lumen of the alimentary tract of sheep V at the time of autopsy

Section of digestive tract	Number of eggs examined		Percentage of eggs in 1-4 cell stage		Percentage of eggs in 5-8 cell stage	
	<i>N. fil.</i>	<i>N. spath.</i>	<i>N. fil.</i>	<i>N. spath.</i>	<i>N. fil.</i>	<i>N. spath.</i>
S. intestine						
7-12 feet . . . .	94	23	68	61	32	39
19-24 " . . . .	112	28	54	54	46	46
31-36 " . . . .	36	141	53	50	47	50
43-48 " . . . .	135	247	37	32	63	68
Cecum . . . . .	43	194	30	13	70	87

eggs segmented. Therefore while cleavages up to and including the third took place comparatively rapidly, the fourth was delayed for a time at least as long as it took for eggs to be transported from the anterior portion of the small intestine to the exterior in feces.

### The Egg-laying Rate

In Table 1 it is shown that most worms contained an uninterrupted array of cleavage stages. This, it is concluded, indicates that the ovaries had been functioning just prior to sampling, if not continuously, then intermittently at short intervals.

Comparison of the number of eggs at each segmentation stage affords more precise information on the rate of egg production. Among those

TABLE 4.—The mean frequency of the various segmentation stages of eggs in utero. This has been given also as a percentage of the mean frequency of unsegmented eggs

Sheep	No. of worms averaged	Mean frequency of cell classes (Percentages in parentheses)							
		Anterior uterus				Posterior uterus			
		1	2	3-4	5-8	1	2	3-4	5-8
<i>N. filicollis</i>									
I .....	18	11.1 (100)	2.7 (24)	1.8 (17)	1.1 (10)	19.4 (100)	4.0 (21)	0.8 (4)	0.2 (1)
II .....	14	9.1 (100)	1.9 (20)	2.0 (22)	1.4 (15)	15.1 (100)	4.1 (27)	1.7 (12)	0.6 (4)
III <sup>a</sup> .....	31	6.4 (100)	2.0 (30)	2.2 (34)	2.2 (34)	13.2 (100)	3.7 (28)	2.9 (22)	0.6 (4)
IV .....	14	14.6 (100)	3.2 (22)	2.3 (16)	—	21.2 (100)	3.9 (17)	2.2 (10)	—
V S.I. 7-12 feet .	28	8.8 (100)	1.6 (19)	1.2 (14)	0.6 (6)	6.2 (100)	1.4 (22)	1.6 (26)	3.4 (55)
<i>N. spathiger</i>									
I .....	6	11.0 (100)	2.7 (24)	2.2 (20)	4.7 (42)	17.5 (100)	2.8 (16)	2.7 (15)	4.2 (24)
II .....	10	7.4 (100)	2.2 (30)	2.3 (31)	4.7 (64)	14.1 (100)	4.9 (35)	2.3 (16)	2.3 (16)
IV .....	4	9.2 (100)	2.2 (24)	2.2 (24)	6.5 (70)	15.5 (100)	3.5 (23)	4.0 (26)	1.2 (8)
V S.I. 7-12 feet <sup>b</sup>	5	8.6 (100)	2.0 (23)	2.0 (23)	0.2 (2)	9.8 (100)	1.4 (14)	2.2 (22)	0.8 (8)
" S.I. 37-42 feet	19	8.4 (100)	2.3 (28)	1.3 (16)	1.4 (4)	9.2 (100)	2.5 (27)	1.3 (14)	0.4 (4)
" Cecum <sup>c</sup> .....	6	6.5 (100)	1.7 (26)	1.5 (23)	1.5 (23)	7.8 (100)	2.3 (30)	1.7 (21)	2.2 (28)

<sup>a</sup> *N. spathiger* absent.

<sup>b</sup> S.I. 7-12 feet, etc.: section of small intestine measured from anterior end.

<sup>c</sup> *N. filicollis* not found in cecum.

worms in which there was an uninterrupted array of segmentation stages it was the rule in individual uteri for the mean number of eggs at particular cleavage stages, earlier than the maximal (i.e., the stage laid by the worm), to occur in a nearly constant ratio (Table 4).

In particular this applied to unsegmented and 2-cell eggs, the positive correlation between which is shown in Fig. 2. The individual fluctuation in the ratio is evident. It is concluded that, where the values for the ratio approached the mean, they were indicative of uniformity in the rate of

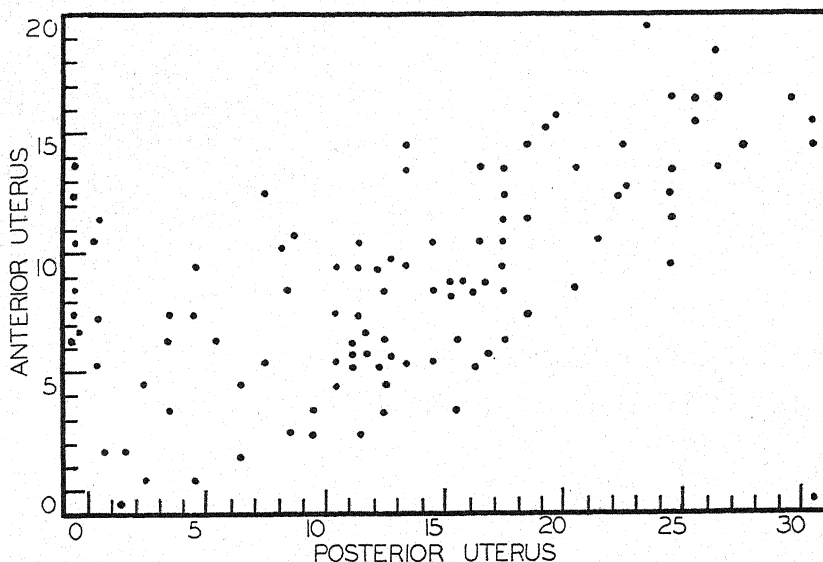


FIG. 1. The correlation in function between ovaries of the same individual of *Nematodirus filicollis*. The number of unsegmented eggs in one uterus, taken as an indication of this, has been plotted against the number in the other uterus of the same worm. In Table 4 the correlation between mean values for different sheep of each segmentation stage is given.

egg-laying on the part of individuals for at least a short time before sampling. A further conclusion is drawn that the differences between uteri in numbers, of a particular segmentation stage, indicated relative fecundity. For the reason that unsegmented eggs outnumbered 2-cell eggs the former are regarded as the more delicate index of this function. Obviously the sum of the numbers of unsegmented eggs for both uteri indicated the relative egg output for individual worms.

In Fig. 1, in which the numbers of unsegmented eggs of the anterior and the posterior uteri are plotted against one another, it is seen that there was positive correlation. Therefore, it follows that the uteri of worms did not alternate in function and that there was a direct relation in their relative fecundity. Table 5 shows that in sheep I, II, III, and IV the posterior ovary, by this same criterion of numbers of unsegmented

TABLE 5.—The frequency with which one or other uterus contained the more unsegmented eggs

Sheep	<i>N. filicollis</i>			<i>N. spathiger</i>		
	Number of worms	Frequency of uterine predominance in unsegmented egg numbers		Number of worms	Frequency of uterine predominance in unsegmented egg numbers	
		Anterior	Posterior		Anterior	Posterior
I .....	(1)	(2)	(3)	(1)	(2)	(3)
II .....	18	1	17	6	—	6
III .....	14 <sup>a</sup>	—	13	10	—	10
IV .....	31	—	31	1	—	1
V S.I. 7-12 feet ..	14	1	13	4	—	4
" S.I. 37-42 feet ..	28 <sup>a</sup>	19	6	5 <sup>a</sup>	—	4
" Cecum .....	—	—	—	19 <sup>a</sup>	5	9
				6 <sup>a</sup>	1	4

<sup>a</sup> The difference between column (1) and the sum of columns (2) and (3) represents the number of worms in which the uteri contained equal numbers of unsegmented eggs.

eggs, produced eggs at a greater rate than the anterior ovary, and that in sheep V, the reverse took place. The direct relation between the two uteri in numbers of unsegmented eggs would mean that for particular conditions the worms functioned to the full extent of their potential.

The number of unsegmented eggs as an index of egg-laying rate is preferable to the total number of eggs found in worms. By the latter method no count is taken of the individual differences in capacity of the individual uteri, nor of time over which the eggs have been accumulated; the possibility of error is therefore greater.

A check on the use of the number of unsegmented eggs in estimating relative egg-laying rates, was obtained from results from the 7-12-foot section of the small intestine of sheep V. By totaling the number of unsegmented eggs of uteri, grouped according to the maximum stage of segmentation they contained, figures were obtained which it is considered indicated the proportional rate at which eggs of a particular stage of segmentation were being laid. Thus among worms of the sample population of *N. filicollis* which contained maximum stages of segmentation of less than four cells there was a total of 301 unsegmented eggs. Where the maximum degree of segmentation was of 5-8 cells, worms contained

TABLE 6.—The frequency with which uteri contained unsegmented eggs

Sheep	<i>N. filicollis</i>				<i>N. spathiger</i>			
	Number of worms	Uteri containing unsegmented eggs			Number of worms	Uteri containing unsegmented eggs		
		Anterior	Posterior	Per cent		Anterior	Posterior	Per cent
I .....	18	17	18	97	6	—	—	—
II .....	14	14	14	100	10	6	6	100
III .....	31	30	31	98	1	10	10	100
IV .....	14	14	13	96	1	1	1	100
V S.I. 7-12 feet <sup>a</sup> ..	28	28	22	89	4	4	4	100
" S.I. 37-42 feet ..	—	—	—	—	5	5	5	100
" Cecum .....	—	—	—	—	19	18	18	95
					6	6	6	100

<sup>a</sup> S.I. 7-12 feet, etc.: section of small intestine measured from anterior end.

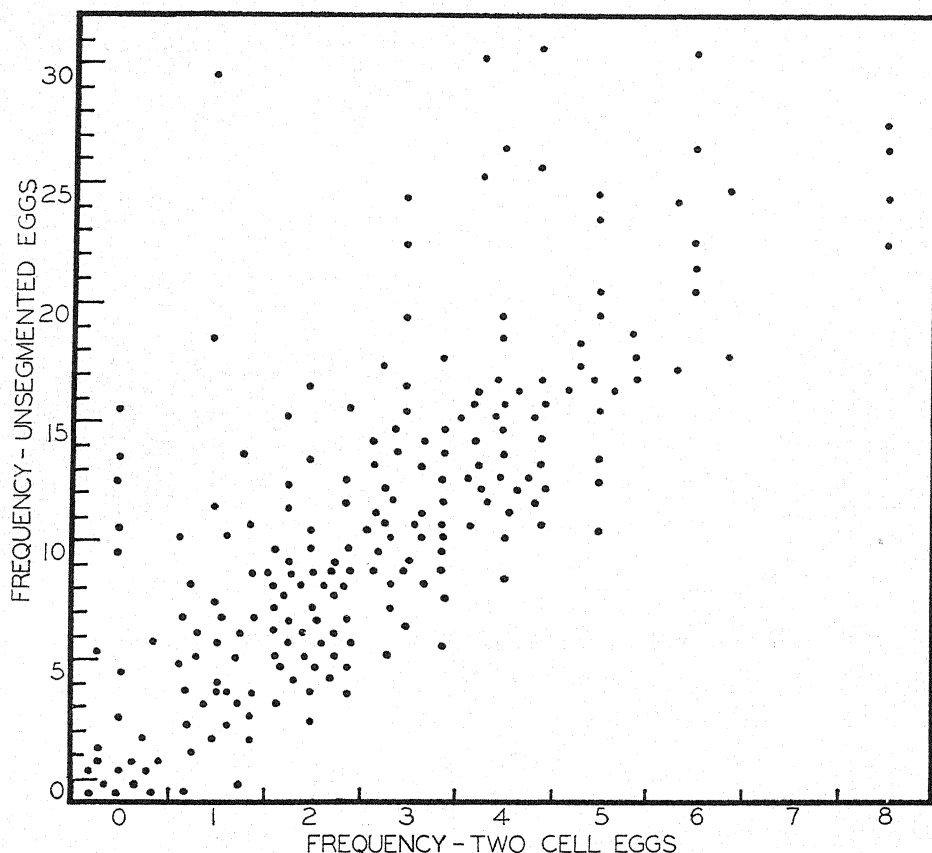


FIG. 2. The number of unsegmented eggs in each uterus of *Nematodirus filicollis* plotted against that of 2-cell eggs in the same uterus. The positive correlation shown is taken as an index of continuity in egg-laying by individuals. See Table 4 for the mean value, for each sheep, of the various segmentation stages.

a total of 100 unsegmented eggs. Comparison of this 3:1 ration was made with that of the same cleavage stages in eggs found free in the lumen of the intestine of this same region (Table 3). Here numbers were respectively 64 and 30, approximately a 2:1 ratio. In view of the small numbers involved and the lag in time between the laying and the examination of eggs found in the lumen the ratios are considered to approximate sufficiently to establish the validity of the numbers of the unsegmented eggs as a measure of the relative egg-laying rates of worms.

On reference to Table 6 it can be seen that among all five sheep the percentage number of uteri which contained unsegmented eggs ranged from 89 to 100 per cent for *N. filicollis*, and from 95 to 100 per cent for *N. spathiger*. These facts, coupled with the previously drawn conclusion that similarity of the ratios of numbers of unsegmented to 2-cell



eggs (Table 4) indicated continuity in egg-laying, lead to the additional conclusion that almost the entire population in each sheep was laying eggs at the time of sampling.

It is noteworthy that in sheep V, from which infection was in process of being eliminated, egg-laying was taking place, though at a retarded rate.

TABLE 7.—The frequency of numbers of unsegmented eggs in utero in populations of *N. filicollis*

Sheep	Class. Number of unsegmented eggs in utero	Frequency		
		Anterior uterus	Posterior uterus	Individual worms
I	0-4	2	2	1
	5-9	4	—	—
	10-14	7	3	1
	15-19	—	5	1
	20-24	5	2	2
	25-29	—	3	3
	30-34	—	3	3
	35-39	—	—	2
	40-44	—	—	2
	45-49	—	—	3
II	0-4	2	2	1
	5-9	5	—	—
	10-14	7	6	1
	15-19	—	2	2
	20-24	—	2	4
	25-29	—	2	1
	30-34	—	—	1
	35-39	—	—	4
III	0-4	6	—	—
	5-9	22	4	2
	10-14	3	14	4
	15-19	—	11	10
	20-24	—	2	7
	25-29	—	—	6
	30-34	—	—	2
IV	0-4	1	1	—
	5-9	—	—	—
	10-14	4	—	1
	15-19	8	3	—
	20-24	1	3	1
	25-29	—	6	1
	30-34	—	1	5
	35-39	—	—	5
	40-44	—	—	4
V	0-4	—	—	1
	5-9	4	13	2
	10-14	11	8	6
	15-19	12	4	8
	20-24	1	3	3
	25-29	—	—	5
	30-34	—	—	3
	35-39	—	—	1
	40-44	—	—	—

The inter-host differences in magnitude of the mean number of uterine unsegmented eggs per individual (Table 4) and the tendency in a population of worms from one host for the uterine number of unsegmented eggs to fluctuate according to the curve of error (Table 1), suggest that the mean value is an index of the particular set of conditions in which the populations were found. It would follow as a corollary that fecal egg numbers also are a measure of these conditions. A further corollary

would be that periodical fluctuations in the fecal egg count have significance in this connection.

In the estimation of the absolute mean fecundity, from fecal counts and the numbers of females found at autopsy, the foregoing indicate that the mean values refer to the modal rate of egg production of the worm population at a particular point of time. Variance in the mean values found for a species, it is concluded, reflects not variance in the numbers of worms which have contributed to the population of eggs, but rather in the particular level for the whole population of the tide of egg production at that time.

#### SUMMARY

A study was made of degree of segmentation and numbers of in utero eggs of *N. filicollis* and *N. spathiger*. It is concluded that:

1. At any one point of time almost the entire population of worms is laying eggs.
2. The numbers of unsegmented eggs in utero indicate the relative fecundity of worms.
3. Among a population of worms the individual fecundity fluctuates about a mean according to the normal curve of error.
4. The absolute mean rate of egg production for individual worms as determined from fecal egg numbers and the population of females found at autopsy, refers to the modal rate of egg production at a particular level in the fluctuating tide of egg production.
5. Fecal egg numbers are an index of the relative behavior of the population as a whole to a particular set of conditions at one point or over a short period of time.

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## EARLY DEVELOPMENTAL STAGES OF STRIGEID TREMATODES IN THE FIRST INTERMEDIATE HOST\*

W. W. CORT AND LOUIS OLIVIER

### HISTORICAL INTRODUCTION

Although the life cycles of more than twenty species of strigeids have been worked out more or less completely, very little is known of the germ cell cycle in this group or of the early stages of development in the first intermediate host. We have found only three studies of strigeid miracidia which give information on the germ cells. Van Haitsma (1930) considered as primordial germ cells a small but variable number of large granular cells which he found in the posterior end of the miracidium of *Diplostomum flexicaudum* (Cort and Brooks, 1928). In one of his figures (Pl. XLV, Fig. 24) he showed four such cells in a row, and labelled them "germ cells." Hunter and Hunter (1935, Pl. C, Fig. 1) depicted a single "germ ball" in the posterior end of the miracidium of *Crassiphiala* (= *Uvulifer*) *ambloplitis* (Hughes, 1927). Park (1936) observed a "germ cell sac" near the posterior end of the miracidium of *Neodiplostomum lucidum* La Rue and Bosma, 1927, which contained a coarse granular fluid and from one to several large floating germ cells. He also regarded as germ cells other large cells lying in the parenchyma anterior to the "germ cell sac."

Looss (1896) seems to have been the first to see a strigeid mother sporocyst. He described and figured (Pl. XV, Fig. 162) this stage of *Cercaria vivax* Sonsino, 1892, which was later shown by Azim (1932) to develop into an adult of the strigeid family CYATHOCOTYLIDAE. Looss also made some very interesting observations on daughter sporocysts of this species. In the youngest daughter sporocysts outside the mother, which were only about 0.3 mm in length, he found two types of germinal elements ("corps germinatifs") free in the body cavity. Some of them were evidently cercarial embryos. The others were irregular structures divided into three or four distinct segments each composed of groups of cells. Looss suggested that these segments probably separated to form cercarial embryos. In the figures of an older sporocyst in which cercariae were beginning to be differentiated he also showed several of these compound structures (Pl. XV, Fig. 165).

Sewell (1922, p. 284) reported the finding of mother sporocysts of *Cercaria indicæ* XV, a form closely related to *C. vivax*. The largest were almost 10 mm in length and had a terminal birth pore.

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Mathias (1925) exposed snails to large numbers of the miracidia of *Strigea tarda* [= *Cotylurus cornutus* (Rud.)] and examined them at intervals to follow early development. In this way he obtained early stages of mother sporocysts, which showed the metamorphosis from the miracidia (Pl. II, Fig. 9 a, b, c). He probably had multiple infections since he exposed the snails to large numbers of miracidia. He concluded that the sporocyst that metamorphosed from the miracidium ("sporocyste primitif") produced a number of new sporocysts ("sporocystes primaires") by transverse fission. The latter then invaded the tissues of the snail and gave rise to another generation of sporocysts ("sporocystes secondaires") which in turn produced the cercariae. Mathias believed that mother sporocysts reproduced by transverse fission because they were often divided into segments by constrictions in the wall (Fig. 9 d).

Brooks (1930) included four species of strigeids in the material used in his study of the germ cell cycle of digenetic trematodes. From the examination of sections of young daughter sporocysts he concluded that the germ cells ("antecedent germ cells") lie free in the body cavity. According to his view these "antecedent germ cells" form "germ masses," which are rather loosely associated groups of undifferentiated cells. These masses later separate into their individual cells ("excomponents") each of which produces a cercarial embryo. He interpreted this method of reproduction as a "specific polyembryony or blastotomy."

Wesenberg-Lund (1934) found the mother sporocysts of five different species of strigeids in examinations of naturally infected snails. They were present along with fully developed daughter sporocysts containing mature cercariae and were all old and almost empty. He recognized them as mother sporocysts from their large size and because they contained a few small daughter sporocysts. They varied considerably in length in the different species and some of them were divided into sections by constrictions.

Hunter and Hunter (1935) found structures which they interpreted as mother sporocysts in snails naturally infected with *U. ambloplitis*. These structures were only 0.2 to 0.3 mm in length and had definite eyespots.

#### MATERIALS AND METHODS

Most of the studies reported in this paper were made on strigeid infections found in juveniles and adults of *Stagnicola emarginata angulata* (Sowerby) collected in the Douglas Lake region during the summer of 1940. In the examination for infections the bodies of the snails were carefully removed from the shells and observed with a low power binocular microscope. If no sporocysts were seen on the surfaces of the organs, we then teased the tissues apart with dissecting needles, looking most carefully in the region between the digestive gland and the foot. When



a mother sporocyst was found, we tried to remove it unbroken for measurement and study while still alive. Daughter sporocysts were studied in the same way. Several *intra vitam* stains, particularly neutral red, were tried but in most cases the details could be worked out best from unstained specimens mounted in normal saline.

Some of the infections, although obviously strigeids, could not be specifically identified because of their immaturity. In other cases, however, we were able to study mother sporocysts and young daughter sporocysts from infections in which some of the cercariae were mature enough for identification.

#### DATA FROM THE OBSERVATIONS

*Mother sporocysts:* A total of 21 strigeid mother sporocysts from naturally infected juveniles and adults of *S. emarginata angulata* were studied during the summer of 1930. Ten were in infections too immature for identification; six belonged to *Diplostomum flexicaudum* (Cort and Brooks, 1928); two to *Cercaria laruei* Cort and Brooks, 1928; two to *C. emarginatae* Cort, 1917; and one to *C. dohema* Cort and Brackett, 1937. In addition, a mother sporocyst was recovered on November 5, 1940 from a laboratory raised juvenile of *S. emarginata angulata* which had been exposed 25 days before to miracidia of *Diplostomum micradenum* (Cort and Brackett, 1938). All these mother sporocysts with the exception of one found on the surface of the basal portion of the digestive gland, were laced in among the organs and ducts between the foot and digestive gland. Frequently a portion of the sporocyst projected from the surface of the undissected snail body, but many of them were found only after careful dissection of the tissues under the mantle on the upper side of the foot.

Almost all the mother sporocysts observed had already given off most of their daughter sporocyst progeny. They were sometimes found in infections in which there were mature cercariae. In such cases, the infection would also contain a whole series of immature daughter sporocysts in the various stages of development. A few of the mother sporocysts, however, still contained all or most of their daughter sporocyst brood. When in position in the host all but a few of the very oldest mother sporocysts appeared as inflated sacs of uniform diameter. When freed intact in normal saline they moved actively; but when they were removed to slides for microscopical examination they were easily broken and frequently sections would collapse. Strigeid mother sporocysts have thick walls consisting of a thin cuticula, muscular layers and a single layer of cells bordering on the large body cavity. The anterior end is attenuated and has a terminal birth pore (Figs. 1, 2).

Most of the mother sporocysts were considerably larger than the daughter sporocysts, especially in width. Four, from infections too im-

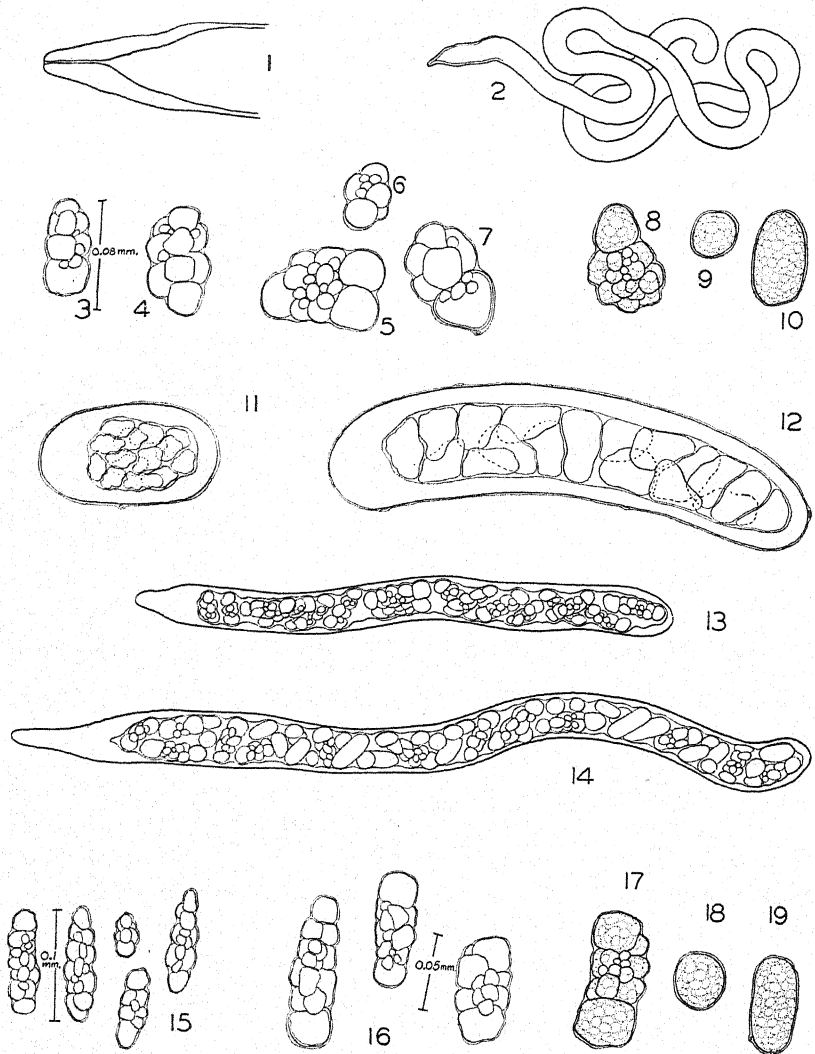


FIG. 1. Anterior end of mother sporocyst of *D. flexicaudum* showing birth pore.

FIG. 2. Complete mother sporocyst of *D. flexicaudum*.

FIGS. 3-7. Outline drawings of germ masses from strigeid mother sporocysts. All drawn to same scale.

FIG. 8. Germ mass from mother sporocyst showing large and small multicellular components and also single-celled components.

FIG. 9. Very young daughter sporocyst embryo (a germ ball). Note similarity to largest component of germ mass (Fig. 8).

FIG. 10. Older daughter sporocyst embryo drawn to same scale as Fig. 9, showing elongation.

FIG. 11. Daughter sporocyst embryo, 0.075 mm long. Note germ masses in body cavity, each composed of several cells.

FIG. 12. An older daughter sporocyst embryo, 0.2 mm long. Note germ masses in body cavity which already have many celled components.

mature for identification, were 5, 7, 10, and 12 mm long. Those of *D. flexicaudum* measured up to 10 mm in length and varied from 0.13 to 0.32 mm in diameter. Two of *C. laruei* measured 17 and 25 mm in length and one of *C. emarginatae* was about 8 mm long and 0.3 mm wide. Except for the larger size in *C. laruei* no specific differences were observed among the mother sporocysts of the five species. Differences, however, will probably be found when more material becomes available for comparative study. Mother sporocysts live and continue to reproduce for a considerable period of time (at least five or six weeks and probably longer) since they were frequently found in infections from which large numbers of cercariae were escaping.

*Contents of mother sporocysts:* In most of the mother sporocysts elongate slender daughter sporocysts were found which appeared to be about ready to emerge. There were also present the various developmental stages of the daughter sporocysts, and a few peculiar structures, which could be easily distinguished from the daughter sporocyst embryos by their irregular outline. They will be referred to as germ masses, using the term employed by Brooks (1930) for similar structures in daughter sporocysts.

*Germ masses:* The germ masses found inside the mother sporocysts were few in number. They varied in length from about 0.05 to 0.12 mm and were composed of a variable number of components all enclosed by a thin membrane (Figs. 3-7). The largest components which were usually at the ends were composed of a mass of cells and were completely surrounded by their own membrane. In a few cases the large end components were seen partly split off from the rest of the germ mass. The germ masses also contained smaller components composed of cell groups and a few distinctly separated single cells near the middle of the mass (Fig. 8).

*Daughter sporocyst embryos:* The youngest daughter sporocyst embryos (Fig. 9) were small spherical structures about 0.025 mm in diameter composed of a number of cells and enclosed by a thin membrane in which flattened nuclei could sometimes be distinguished. They were

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FIG. 13. Daughter sporocyst (embryo) 0.8 mm long from mother sporocyst. Note that only germ masses are present in the body cavity.

FIG. 14. Daughter sporocyst dissected from snail tissue and evidently recently emerged from the mother. Note many germ masses but also a number of very young cercarial embryos.

FIG. 15. Outline drawings of germ masses from daughter sporocysts before they emerged from the mother. Drawn to same scale.

FIG. 16. Outline drawings of germ masses from young daughter sporocysts dissected from snail tissue.

FIG. 17. Germ mass from daughter sporocyst showing large and small multicellular components and also single celled components.

FIG. 18. Very young cercarial embryo. Note similarity to larger components of germ mass (Fig. 17).

FIG. 19. Cercarial embryo slightly older than in Fig. 18.

about the same size and had exactly the same structure as the largest components of the germ masses. A daughter sporocyst embryo in slightly later stage, which was more elongate but in which no differentiation of the cells could yet be distinguished, is shown in Fig. 10. In daughter sporocyst embryos about 0.05 mm long the body wall could be first seen as separate from the germinal material in the cavity; and at a somewhat later stage, about 0.07 to 0.08 mm long, the wall was very clearly separated from the contents of the body cavity (Fig. 11). The body wall in these early daughter sporocyst embryos was comparatively thick and consisted mainly of the single layer of large cells lining the cavity. The germinal material at this stage was organized into a number of germ masses, the components of which appeared to be single cells. An older stage (Fig. 12), about 0.2 mm in length, contained germ masses in which some of the components already consisted of cell groups. Still older daughter sporocyst embryos were studied in a 25-day-old mother sporocyst of *D. micradenum* which developed from an experimental infection. About 20 daughter sporocysts were observed in this mother, none of which appeared to be ready to escape. Seven that were measured varied from 0.31 to 0.46 mm in length and from 0.03 to 0.05 mm in width. The numbers of germ masses they contained could be easily counted and varied from 12 to 19.

*Daughter sporocysts ready to emerge from the mother* (Fig. 13): Daughter sporocysts which appeared to be about ready to emerge from the mother were quite mobile and had a considerable power of extension and contraction. They varied in length from about 0.6 to 1.0 mm and were sometimes as much as 0.1 mm in diameter. The anterior end of the daughter sporocysts at this stage was attenuated and very mobile and the body cavity did not extend into it. No trace of the birth pore could be seen. A careful examination disclosed that the body wall was composed of a thin outer cuticula, muscular layers, and a single layer of cells bordering on the body cavity. In the body cavity were a number of germ masses which did not fill the cavity and which moved back and forth in it with the movement of the sporocyst. Sometimes the germ masses were crowded into less than half the length of the cavity. The number of germ masses in a series of 18 of these daughter sporocysts varied from 13 to 23 with an average of 17. The material examined was not sufficient to determine whether the number of germ masses varies with the species. These germ masses varied in length from 0.02 mm to almost 0.12 mm. While essentially like those found in the mother sporocysts, they were generally somewhat smaller and tended to be more elongate. They had a variable number of components, the typical arrangement of which is shown in Fig. 15. Single cells were never found in the body cavity of daughter sporocysts. In only three cases were early cercarial embryos seen in daughter sporocysts still within the

mother. One of these was a daughter sporocyst of *D. flexicaudum*, 0.8 mm in length, which contained, in addition to 20 germ masses, four cercarial embryos. Also, in two daughter sporocysts of *C. emarginatae* which were still within the mother there were two and six of these very young cercarial embryos. These findings and the constant presence of cercarial embryos in the youngest daughter sporocysts found outside the mothers, indicate that the first cercarial embryos are just beginning to appear at the time of emergence of the daughters from the mother.

*Daughter sporocysts from host's tissues:* In young infections the immature daughter sporocysts may all be located among the organs in front of the digestive gland; some, however, always reach this gland rather early in the development of the infection. In older infections in which some cercariae have reached full development, the daughter sporocysts will have invaded almost the whole of the digestive gland, but numbers of them remain between the foot and this gland. In a number of cases the mother sporocysts still contained a few daughters after the infection had reached maturity. In such infections a whole series of the different developing stages of daughter sporocysts would be found in the snail's tissues, including some about the same size as those still contained in the mother.

Most of the youngest daughter sporocysts outside the mothers showed an increase in size (Fig. 14). In their body cavities mixed in with the germ masses were varying numbers of the youngest cercarial embryos (Fig. 18). They were very much like the youngest daughter sporocyst embryos and were also about the same size and of exactly the same structure as the largest end components of the germ masses (cf. Figs. 17 and 18). Each consisted of a spherical mass of apparently undifferentiated cells surrounded by a thin membrane containing a few flattened nuclei. These youngest cercarial embryos increased considerably in size while still appearing as a mass of cells (Fig. 19). Soon the individual cells could not be seen and the size increased further. Later the differentiation of body and tail occurred. As the daughter sporocysts increased in size, the number of developing cercarial embryos rapidly increased but the number of germ masses apparently remained constant.

A series of counts of the germ masses and cercarial embryos was attempted in young daughter sporocysts of different sizes dissected from the tissues of the snails. One unidentified daughter sporocyst 1.3 mm in length contained 18 germ masses and 24 cercarial embryos, the largest of which was only about 0.06 mm long as compared with 0.12 mm for the largest germ mass. In a sporocyst of *C. laruei*, 1.34 mm in length, there were 18 germ masses and about 40 cercarial embryos some of which showed considerable differentiation; and in a sporocyst of *C. emarginatae* of about the same size there were 17 germ masses and 25 very young cercarial embryos. The contents of three unidentified daughter sporocysts



1.8 to 2.0 mm in length were also counted. They contained 20, 26, and 16 germ masses respectively and about 50, 93, and 100 cercarial embryos, the oldest of which had not yet reached the stage of tail differentiation. In a few larger sporocysts up to over 4 mm in length counts were made which gave the impression that even at the stage where the cercariae show a well differentiated body and tail there was no reduction in the number of germ masses. Fig. 16 shows germ masses from the young daughter sporocysts after their escape from the mother.

In a considerable number of mature and old daughter sporocysts of *D. flexicaudum*, *C. laruei*, *C. emarginatae*, and *C. yohana* Cort and Brackett, 1937, from 2 to 10 germ masses were still found, scattered among cercarial embryos in all stages of development. We could not be certain that there was really a reduction in the numbers of germ masses in such sporocysts because the numerous cercarial embryos tended to obscure them. Germ masses and immature cercarial embryos were even found in sporocysts so old that they were almost entirely empty. The germ masses found in mature and old sporocysts differed little from those in the immature sporocysts. In 12 such germ masses the number of multicellular components varied from 4 to 9 and the single celled components from 2 to 5. We have gained the general impression from these observations that there are fewer germ masses in the oldest sporocysts and that those that are left have somewhat fewer components. The most significant point, however, is that even in old sporocysts, which are partly empty and beginning to degenerate, germ masses and very immature cercarial embryos may still be found.

*Numbers involved in development:* We obtained a little information on the number of daughter sporocysts and cercariae normally produced from a single miracidium. In one case more than 50 daughter sporocysts in addition to some germ masses and developing embryos were found inside a mother sporocyst; and in another case over 60 daughter sporocysts were dissected from the tissues of a juvenile snail. Although it will have to be checked by later studies, we have the general impression that the number of daughter sporocysts produced by a single mother may be considerably larger. Finally, in one daughter sporocyst 8.7 mm in length in which none of the cercariae were mature we counted almost 1,000 embryos.

#### DISCUSSION

The data from our investigations given above make it possible to understand more clearly some of the phases in the life cycle of the strigeids. On the basis of the descriptions of Van Haitsma (1930), Hunter and Hunter (1935), and Park (1936) it appears that strigeid miracidia contain only a small number of germ cells. It is suggested that each of these germ cells develops into a germ mass when the miracidium metamorphoses into the mother sporocyst. Our observations indicate to us

that these germ masses give rise to the daughter sporocyst embryos by splitting off their larger components and are the only source of daughter sporocyst embryos. The striking similarity between the youngest daughter sporocyst embryos and the largest of the germ mass components supports that view. Also, the germ masses are loosely organized and in many cases the largest components appear to be ready to split off or are even occasionally partly separated from the rest of the mass. Evidently daughter sporocyst embryos begin to split off from the germ masses rather early in the development of the mother sporocysts. This would account for the production of the first mature cercariae in about six weeks after the penetration of the miracidium into the snail (Van Haitsma, 1930; Mathias, 1925). New daughter sporocyst embryos are split off over a considerable period of time, probably a month or even more. The evidence for this is that mother sporocysts which were obviously old and which had produced daughters which were already mature contained germ masses and young daughter sporocyst embryos. To make possible this long period of production of daughter sporocysts it is evident that the cells of the germ masses must continue to divide and to produce new components as the largest ones break off.

We did not find the slightest evidence in our studies that mother sporocysts reproduce by fission or fragmentation as suggested by Mathias (1925).

Our studies also suggest that very early in the development of the daughter sporocyst embryo a small number of cells of the germinal line come to lie in the cavity surrounded by the soma and that each of these develops into a germ mass. We believe that these germ masses of the daughter sporocysts are the only source of cercarial embryos and give rise to them by the splitting off of the largest components. There is considerable evidence for this view. In the first place, germ masses are the only structures we have seen in the body cavities of the developing daughter sporocysts up until about the time that they emerge from the mother; we have constantly looked for single germ cells but have never found them. Also, the youngest cercarial embryos are strikingly like the largest end components of the germ masses. Finally, it has been observed that the largest components may be loosely attached to and even partly separated from the rest of the germ mass and appear to be ready to break off.

As suggested for the germ masses of the mother sporocysts, the single cell elements of the germ masses of the daughters must continue to divide and produce new multicellular components to take the place of those that are split off. The best evidence that such division does occur comes from the fact that the germ masses of old daughter sporocysts in which large numbers of cercariae have been produced still have single-celled components. Also, the total number of components in the germ masses

found in a young daughter sporocyst is much smaller than the number of cercariae usually produced by each daughter.

The most significant contribution made in this study is the description of the germ masses of the mother and daughter sporocysts and the elucidation of the method of production of the daughter sporocyst and cercarial embryos. It is not at all surprising that germ masses have not been recognized previously in mother sporocysts since so little attention has been paid to this phase of the strigeid life cycle. Perhaps the single "germ ball" that Hunter and Hunter (1935, Pl. C, Fig. 3) figured in the posterior end of the mother sporocyst of *U. ambloplitis*, and the groups of cells shown by Mathias (1925, Pl. II, Fig. 9) in the end segment of a mother sporocyst of *C. cornutus* were really germ masses.

On the other hand, the germ masses of the young daughter sporocysts of strigeids are such conspicuous and unusual structures and so different from all the stages of the cercarial embryos, that it is surprising that they have not been observed more often. As noted above Looss (1896) described what appear to be germ masses in his studies of young sporocysts of *C. vivax*. He even suggested that cercarial embryos were produced by the separation of the multicellular components of these structures. Also, the "germ balls" figured by Mathias (1925, Pl. III, Fig. 4) in the body cavity of a young sporocyst were undoubtedly germ masses.

Brooks (1930) came the closest to a correct interpretation of the part played by the germ masses in strigeid development. He interpreted them as masses of cells formed by the division of the original germ cells ("antecedent germ cells"). According to his view the cercarial embryos were formed by the breaking up of these masses into single-celled "ex-components" each of which formed a cercarial embryo. He described and figured single cells, which he interpreted either as "antecedent germ cells" or "ex-components" in the body cavities of young strigeid daughter sporocysts. We have found only germ masses or cercarial embryos, never single cells, in the body cavities of the young living daughter sporocysts we have observed. This difference in observations was not due to the use of different material, since two of the species Brooks studied, *C. laruei* and the cercaria of *D. flexicaudum*, were also studied by us. It may be significant in this connection to note that when daughter sporocysts were kept too long in normal saline, the youngest cercarial embryos tended to disintegrate, freeing numbers of single cells. Since Brooks made all his studies on fixed and sectioned material, it may be that some of the embryos or germ masses disintegrated during fixation or manipulation. It is also significant that the two strigeid germ masses which Brooks figured (Pl. III, Figs. 40 and 42) clearly show many-celled components and that he suggested that these components were the early stages of cercarial embryos, which had precociously developed before the single-celled components were separated from the germ mass.

Most of the recent studies of the germ cell cycle of digenetic trematodes support the theory of "germinal lineage" (Dollfus, 1919; Brooks, 1930; Cable, 1934; and Chen, 1937). According to this view reproduction in the first intermediate host is brought about by the segregation of germ cells, which early in embryonic development come to lie in the body cavities of the sporocysts or rediae. These germ cells multiply and eventually give rise to numerous progeny apparently without undergoing maturation or fertilization. Our work on the early developmental stages of the strigeids fits in with this view. In this group, however, a great multiplication of individuals is brought about by the development from the germ cells of a limited number of discrete structures, the germ masses, which produce numerous progeny over a considerable period of time. This development of daughter sporocyst and cercarial embryos by the splitting off of components from the germ masses may perhaps be considered as a special type of polyembryony.

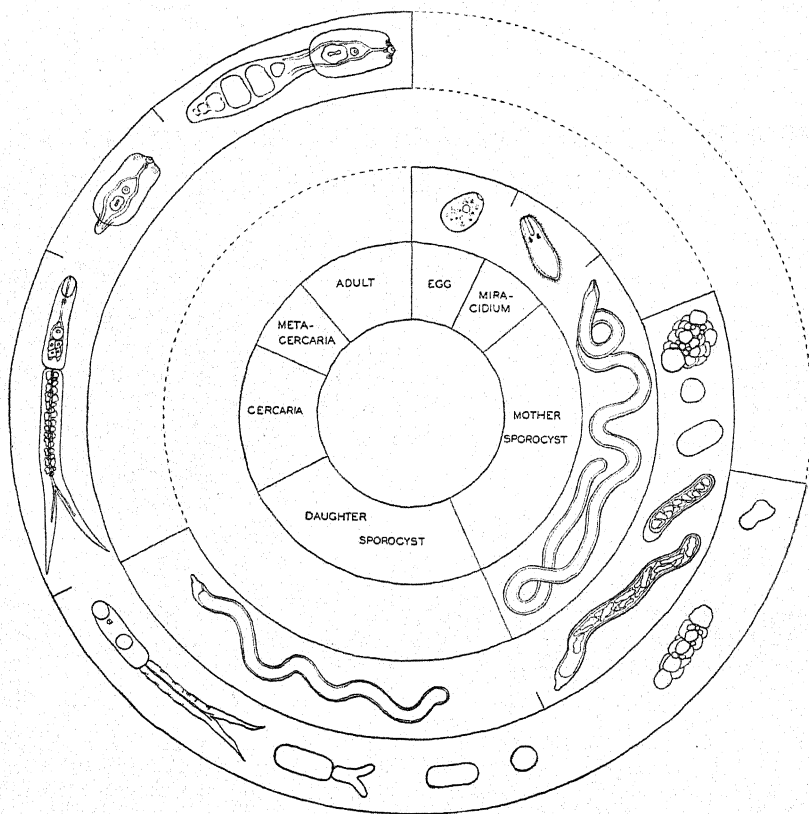


FIG. 20. Diagrammatic outline of the life cycle of a strigeid, *Diplostomum flexicaudum*.

## SUMMARY OF THE LIFE CYCLE IN THE STRIGEIDS

From the evidence given in the preceding discussion, the life cycle in the strigeid group may be considered as a succession of three generations in two of which there are phases of reproduction by a special type of polyembryony (see Fig. 20). The first generation begins with the fertilized ovum produced by the adult, includes the free living miracidium, and ends with the mother sporocyst parasitic in the first intermediate host. The second generation begins with the few germ cells set aside in the development of the miracidium which produce the germ masses found in the mother sporocyst. These germ masses give rise to the daughter sporocysts which leave the mother sporocyst to finish their development in the tissues of the first intermediate host. The third generation starts with a small number of germ cells separated from the soma early in the development of the daughter sporocyst embryo. These produce germ masses, the end components of which begin to be freed as cercarial embryos at about the time the young daughter sporocysts escape from the mother. New cercarial embryos continue to be formed in the same way during the whole life of the daughter sporocysts. The cercariae escape from the snail host and penetrate into a second intermediate host where they go through a long period of metamorphosis to produce the fully developed metacercariae. These, after transfer to the definitive host, develop in a comparatively short time into adults.

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A REDESCRIPTION OF *CONTRACAECUM MULTIPAPILLATUM* (VON DRASCHE, 1882) (NEMATODA: ANISAKINAE)

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In this paper the writer presents a redescription of *Contracaecum multipapillatum* because it is evident from the literature (1) that the species concept of von Drasche (1882) needs more detailed and complete definition and (2) that the description by Skrjabin (1916) of an entirely different species as identical with *Ascaris multipapillata* has resulted in confusion as to the morphological characteristics of von Drasche's species.

The specimens which the writer regards as identical with *A. multipapillata* von Drasche, 1882 and upon which this redescription is based were examined in connection with a study of representatives of the subfamily ANISAKINAE in the Helminthological Collection of the U. S. National Museum.

In addition to the following review of the literature and redescription of von Drasche's species, this paper includes a proposal of a new name for the specimens which the writer regards as misidentified by Skrjabin.

REVIEW AND CRITIQUE OF THE LITERATURE

Von Drasche's (1882) description of *Ascaris multipapillata* included no statements as to the nature of the digestive tract and was incomplete in several other respects, but was sufficient to permit recognition of the species. Stossich (1896) mentioned the species but added nothing concerning its morphology. As has been noted, Skrjabin (1916) published a detailed description of "*Contracoecum multipapillosa* (Drasche 1882)". Obviously, Skrjabin considered that he had redescribed *Ascaris multipapillata* and had allocated the species, on the basis of the structure of the specimens before him, to the genus *Contracaecum* Raillet and Henry, 1912. However, in the specimens from *Ardea* sp. described by Skrjabin, interlabia were absent, whereas von Drasche described and figured interlabia in *Ascaris multipapillata*. Skrjabin stated that 10 pairs of postanal papillae were present in his specimens, 7 pairs being lateral in position and 3 pairs submedian. He did not mention the presence of double papillae. His illustration of the lateral aspect of the male tail (Pl. 24; Fig. 8), according to the legend pertaining to it, indicated the presence of 7 pairs of lateral postanal papillae; evidently, the submedian papillae were intentionally omitted from the figure. Von

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Drasche (1882) stated that 10 postanal papillae (obviously from his figure, 10 pairs) are present in the male of *A. multipapillata*; he noted that papillae 5-10 are arranged in two rows behind the anus and that papillae 5 and 6 may be regarded as a double papilla. In one specimen, von Drasche found a third papilla united with these two on one side of the tail. His figure showed that 2 of the 4 pairs of papillae in the subterminal group were lateral or sublateral in position; possibly, 2 pairs of the single papillae just behind the cloaca might be considered sublaterals, but their position ordinarily would be regarded as subventral. Hence von Drasche figured 8 pairs of subventral and 2 pairs of lateral postanal papillae in *A. multipapillata*. This arrangement differs markedly from that described by Skrjabin.

It should be noted that Skrjabin's illustration (Pl. 24; Fig. 8), contrary to his statements in the legend and text, appears to show that the 7 postanal papillae figured actually were subventral rather than lateral in position. However, since the remaining 3 of the 10 postanal papillae reported to be present were not figured, the only existing evidence as to their position is Skrjabin's statement that it was submedian. Therefore, it cannot be shown on the basis of any justifiable interpretation of the data given by Skrjabin that the postanal papillary pattern of his specimens was identical with or closely resembled that figured for *A. multipapillata* by von Drasche. Finally, the fact that Skrjabin's specimens were reported to lack interlabia leaves no alternative but to conclude that they were of a species distinct from von Drasche's. Although Skrjabin, therefore, did not clarify the structure, or establish the systematic position of *A. multipapillata*, he could have been credited with the transfer of the species to *Contracaecum* had his spelling of the specific name corresponded to that of von Drasche.

In the same year, Geddoelst (1916) assigned "*Ascaris multipapillata* Drasche 1882" to *Kathleena* Leiper and Atkinson, 1914, without, however, combining *Kathleena* and *multipapillata*. Baylis (1920) synonymized *Kathleena* with *Contracaecum*, and tentatively included in *Contracaecum* all of the species referred to *Kathleena* by Geddoelst; he also made the combination "*Contracaecum multipapillatum* (v. Drasche, 1882)". As has been previously stated, von Drasche did not describe the digestive tract of *Ascaris multipapillata* and, so far as is evident from their papers, neither Geddoelst nor Baylis had any knowledge concerning its structure. Actually it is of the *Contracaecum* type. Thus, Baylis (1920) receives credit for the transfer of von Drasche's species to *Contracaecum* for he first made the proper combination.

Later writers have referred to *C. multipapillatum*, but have contributed no information as to its morphology with one probable exception. Walton (1923) mentioned the species in discussion and Layman

and Mudretsova (1926) included "*C. multipapillata*" in their key to species of the genus. It is evident from this key that the Russian authors had in mind the specimens described by Skrjabin (1916), not *C. multipapillatum* (von Drasche). Cram gave an account of the morphology of "*Contracaecum multipapillatum* (Drasche, 1882) Baylis, 1920b"; it combined data and illustrations taken from both von Drasche and Skrjabin and, therefore, must be regarded as actually relating to two species. Witenberg (1929) mentioned "*C. multipapillosum* (Drasche)", but apparently his remarks were based on Cram's account. Hsü (1933) described the esophageal glands of "*Contracaecum multipapillatum* (Drasche, 1882)". While Hsü's paper contained no reference to specific characters, there is no reason to question his identification and it may be presumed that his work first established that the *Contracaecum*-type esophagus occurs in von Drasche's species. So far as the writer has been able to determine, there have been no other references to *C. multipapillatum*.

*Contracaecum multipapillatum* (von Drasche, 1882) Baylis, 1920

**Synonymy:** *Ascaris multipapillata* von Drasche, 1882; *A. multipapillosa* Skrjabin, 1916 [= *A. multipapillosa* Drasche 1882 of Skrjabin (1916)]; nec *Contracaecum multipapillosa* Skrjabin, 1916 [= *Contracoecum multipapillosa* (Drasche 1882) of Skrjabin (1916)].

**Description:** In median optical section of body, edges of cuticular striae in region just behind lips serrated (Figs. 1, 2, 3); diameter of head less than diameter of body just behind lips. Lips widest near anterior extremities and narrowest at base; height and greatest breadth about equal. Anterior margin of pulp only slightly indented medially and antero-lateral margins more or less rounded when outer surface of lip is viewed (Fig. 3); cuticle extending beyond pulp anteriorly and inwardly toward axis of mouth, medially indented forming two lobes (Figs. 3, 5); cuticle also extending beyond pulp in antero-lateral regions forming more or less triangular wing-like projections (Figs. 3, 5). From angle of greatest lateral expansion outline of cuticular projections passing toward median line intersecting pulp near location of cephalic papillae then curving posteriorly concavely to origin (Fig. 3). Pulp indented just behind location of papillae; outline then curving convexly to posterior origin. Internal, principally cuticular, portion of lip narrowest where pulp is widest (Fig. 3). In en face view of lips, pulp divided into an external and internal section near base of lips; external section extending farther anteriorly than internal section, but neither extending into anterior internal lobes of lip or extremes of antero-lateral projections (Fig. 5). When lip is viewed from outer surface, the pulp forms two lateral lobular areas of greatest density which are widest in region beneath papillae and taper posteriorly. Interlabia robust in median longitudinal section (Fig. 3), nearly as high as lips; broad at base, curving inward toward mouth axis, tapering anteriorly to rounded tip; roughly triangular in outline when viewed from external surface (Fig. 1). Muscular esophagus long and slender; diameter almost uniform. Esophageal ventriculus small, oval, width greater than length. Esophageal appendix short, slender, typically 1/7 to 1/8 as long as esophagus. Intestinal cecum extending almost to position of nerve ring; voluminous at origin, tapering to an anterior tip about equal in diameter to esophagus. Cecum roughly 4/5 as long as esophagus (Fig. 2). Lateral cervical papillae sessile, near level of nerve ring.

The principal size relationships of 2 female and 5 male specimens are shown in Table 1.

TABLE 1.—Principal size relationships of *Contracecum multipapillatum*.  
(All measurements in mm.)

	Females		Males				
	1	2	1	2	3	4	5
Body Length .....	35.0	35.0	25.0	18.0	16.0	18.5	12.7
Maximum width .....	1.09	1.0	0.79	0.77	0.83	0.62	0.54
Muscular esophagus Length .....	4.98	4.76	3.84	3.69	3.47	2.80	2.58
Average width .....	0.15	0.15	0.14	0.14	0.17	0.12	0.10
Esophageal ventriculus Length .....	0.14	0.14	0.12	0.08	0.10	0.08	0.06
Width .....	0.19	0.21	0.17	0.16	0.17	0.13	0.08
Esophageal appendix Length .....	0.70	0.65	0.52	0.47	0.43	0.50	0.44
Ratio to length of esophagus .....	1:7.4	1:7.5	1:7.6	1:8	1:8.3	1:5.7	1:6
Intestinal cecum Length .....	4.12	4.19	3.19	2.94	2.72	2.35	2.02
Ratio to length of esophagus .....	4:5	4:4.7	4:5	4:5	4:5.2	4:4.7	4:5.3
Trail Length .....	0.22	0.22	0.14	0.14	0.15	0.14	0.10
Nerve ring Distance from anterior end of esophagus ..	0.37	0.46	0.52	0.37	0.39	0.36	0.30
Vulva Distance from anterior extremity .....	11.71	12.65	.....	.....	.....	.....	.....
Ratio to body length .....	1:3	1:2.8	.....	.....	.....	.....	.....
Spicules Length .....	.....	.....	1.9	1.8	1.9	1.54	0.78

*Female*: Maximum observed length about 35 mm. Vulva near point marking beginning of second third of body length. Tail short, acute, lacking terminal spike or other specialized cuticular or hypodermal processes (Fig. 4). Eggs in utero slightly oval, about 50  $\mu$  by 60  $\mu$ .

*Male*: Maximum observed length about 25 mm. Cuticle of ventral surface in caudal region with numerous longitudinal intrastrial ridges. Tail short, terminating in a rather pointed tip. Preanal papillae about 60 to 70 pairs; each subventral linear series arranged in a single row, except that occasionally a few pairs may form two rows immediately in front of the cloaca. Three or four pairs of adanal papillae present (Figs. 6-7). Postanal papillae usually 11 pairs, typically distributed as follows: A subterminal group of 2 subventral and 3 sublateral pairs; 6 subventral pairs in area between subterminal group and a transverse line through the posterior edge of the cloaca, the arrangement on each side being two papillae united to form a double papilla, one single papilla lateral to postero-lateral and three antero-lateral to double papilla (Fig. 6). The middle pair of the 3 subterminal sublateral pairs may be phasmids. Spicules approximately equal; about 1.7 to 1.9 mm long in apparently fully grown specimens, ranging down to about one-half this length in young specimens.

*Hosts*: *Mycteria americana* (= *Tantalus loculator*); *Anhinga anhinga*; *Ardea herodias*.

*Location*: Esophagus (?) and stomach.

*Distribution*: Brazil; United States (Arkansas, Florida, National Zoological Park, District of Columbia.).

*Specimens*: U. S. N. M. Helm. Coll. 30593; 30600; 31654.

Some of the specimens (U.S.N.M. No. 30593) upon which the above redescription is based were identified by Dr. E. B. Cram. None of the specimens are from the type host of von Drasche's species; however, in position and number the male postanal papillae correspond very closely with von Drasche's description and the lips and interlabia resemble those figured by him. It will be noted that in the females the vulva was in the anterior part of the body; in the specimens which Skrjabin regarded as identical with *C. multipapillatum* (von Drasche) the vulva was situated at the beginning of the posterior third of the body length.

So far as the writer has been able to determine, von Drasche's species is valid. It cannot be denied that no disposition has yet been made of some of the old specific names combined with *Ascaris*, but nearly all of the species which were recognizable from their descriptions now have been redescribed, allocated to proper generic positions, or relegated to synonymy. Thus far, it has not been suggested that *C. multipapillatum* is identical with any species named earlier.

The arrangement of the male postanal papillae in *C. multipapillatum* approaches that described for *C. granulosum* and *C. osculatum*. In one male examined by the writer, the pericloacal papillae formed two longitudinal series on each side of the cloaca much more definitely than is shown in Fig. 6; not only were the 4 single postanal papillae involved, but also the adanal papillae and the first 4 precloacal papillae. Thus, the similarity to the distribution of the pericloacal papillae in *C. osculatum*,



according to the figures of some authors, was striking. However, from a detailed comparison of the writer's specimens with available descriptions of *C. granulorum* and *C. osculatum* it appears that there are constant differences in the number and distribution of the postanal and pre-anal papillae in males of the three species and that there also are other differences of specific value.

According to Cram (1927) and Baylis and Daubney (1922), the male of *C. rosarium* (Connal, 1912) was described as having but three pairs of postanal papillae. Nevertheless, the latter authors identified some immature specimens from *Nycticorax griseus* as Connal's species, although they found 9 pairs of postanal papillae on the tail of an immature male. The arrangement of the papillae in the group just posterior to the cloaca of the male, as figured by Baylis and Daubney, is similar to that found in *C. multipapillatum* and it is quite possible that *C. rosarium* of Baylis and Daubney (1922) may be identical with *C. multipapillatum*.

Hsü (1932) commented on the variation in the number and arrangement of the male caudal papillae in specimens identified by him as *C. spiculigerum*; one of his illustrations (Pl. 1; Fig. 6) of the tail of a male specimen from a cormorant is very suggestive of *C. multipapillatum*. *C. andersoni* Vevers, 1923, also seems to resemble *C. multipapillatum*.

*Contracaecum ainterlabium* n. sp.

It is considered expedient at present, for reasons which cannot appropriately be explained here, to retain in the genus *Contracaecum* the species described by Skrjabin as "*Contracoecum multipapillosum*," although it lacks interlabia and hence does not completely conform to the generic diagnosis, as was noted by Skrjabin. However, a new name must be proposed for the species as will be evident from the following discussion.

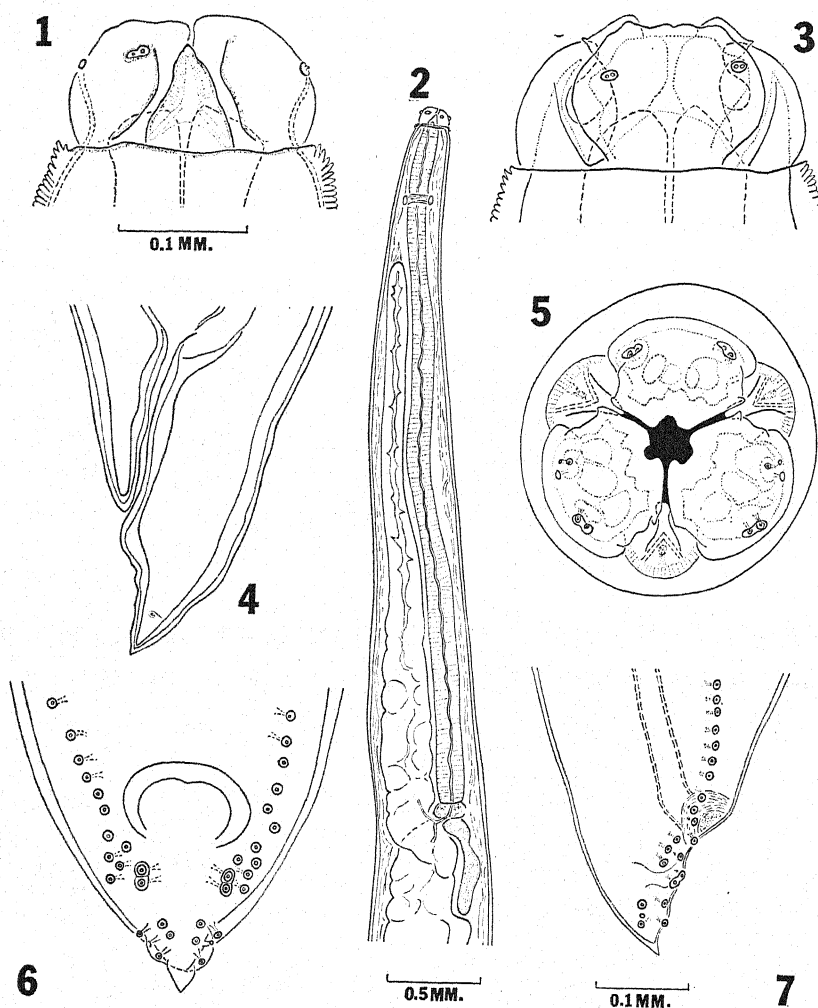
Skrjabin attributed the specific name "*multipapillosum*" to von Drasche in connection with the citation of a reference to the latter's paper and elsewhere he referred to "*Ascaris multipapillosa* Drasche 1882." Although subjectively a mere misspelling is involved, objectively Skrjabin applied a new name to von Drasche's species. The first name applied to a species is the valid name and *Ascaris multipapillosa* Skrjabin, 1916, is a synonym of *Ascaris multipapillata* von Drasche, 1882, as has been indicated elsewhere in this paper. The name *multipapillosa* hence is not available for Skrjabin's species, regardless of generic allocation, because its status is that of a rejected synonym (Internat. Rules Zool. Nomenclature, Art. 36). Skrjabin's species, if valid, is hence unnamed.

Therefore, since so far as can be determined, the species described by Skrjabin (1916) from *Ardea* sp. is not identical with any previously described, the designation *Contracaecum ainterlabium* n. sp. (syn. *Con-*

*tracaeum multipapilloso* Skrjabin, 1916 [= *Contracoecum multipapilloso* (Drasche 1882) of Skrjabin (1916)]; nec *Ascaris multipapilloso* Skrjabin, 1916 [= *Ascaris multipapilloso* Drasche 1882 of Skrjabin (1916)]; nec *Ascaris multipapillata* von Drasche, 1882) is proposed for it.

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*Contracaecum multipapillatum*

FIG. 1. Latero-dorsal view of head of male specimen showing shape of interlabium.

FIG. 2. Lateral view of anterior region of male specimen showing muscular esophagus, esophageal ventriculus and appendix, and intestinal cecum.

FIG. 3. Dorsal view of head of female specimen showing shape of dorsal lip and of interlabia; to simplify the figure the outlines of the subventral lips are omitted. (Same scale as Fig. 1.)

FIG. 4. Lateral view of posterior extremity of female specimen. (Scale one-half that of Fig. 1.)

FIG. 5. *En face* view of head of male specimen. (Same scale as Fig. 1.)

FIG. 6. Ventral view of tail region of male specimen showing postanal, adanal, and a few preanal papillae. (Scale one-half that of Fig. 1.)

FIG. 7. Sublateral view of tail region of male specimen showing postanal, adanal, and a few preanal papillae.

## THE ABSENCE OF OPALINIDS FROM THE ADULT GREEN FROG, *RANA CLAMITANS*

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Opalinas do not ordinarily occur in the adult green frog *Rana clamitans*. In examinations of over 200 green frogs I have not found them harboring infections of opalinids. This absence has been discussed by Metcalf (1923), Van Orden and Nelson (1926), and Hegner (1932). Metcalf (1923) states that possibly the opalinids are adapted only to the tadpole stage; that since this species of frog remains for two years in the tadpole stage, the cysts are passed from tadpole to tadpole. Van Orden and Nelson (1926) have suggested that opalinids have only recently entered *Rana clamitans* and are only in the process of adaptation to the adult stage of this frog. Hegner (1932) suggests that digestive secretions of *Rana clamitans* render the rectum of the adult frog unfavorable to opalinids.

In the present investigation, experiments were conducted to determine if opalinids would live when transferred to adult *Rana clamitans* by cloacal injection. Transfers were made in a solution of Pütter's fluid with powdered egg albumen by means of a small pipette. Injections were made into the recta of 20 frogs.

In no case were infections found later than 5 days after inoculation. In the majority of cases the opalinids disappeared 3 days after inoculation. These results agree with Hegner (1932) who, using similar methods, recovered a few opalinids from the rectum of 4 specimens of *Rana clamitans* 2 or 3 days after inoculation. On the contrary, Van Orden and Nelson (1926) found good infections of *Opalina* in two specimens of *Rana clamitans*, 162 and 174 days respectively after inoculation. It is interesting to note that these men treated the rectal contents with magnesium chloride before inoculation. Trichomonads and the fluke *Dip-lodiscus temperatus* which are often present in frogs would have been removed by this treatment as results in this laboratory show. These parasites were not mentioned by the above authors.

### *Trichomonas* IN RELATION TO *Opalina*

In view of the results obtained by Van Orden and Nelson, studies were made of the incidence and number of *Trichomonas* in 19 species of amphibia. It was found that *Trichomonas* was a common inhabitant of the recta of amphibia in general, and that it was especially abundant in

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the green frog, *Rana clamitans*, as well as in various salamanders. Examination of over 100 green frogs, and 87 salamanders showed their recta to be swarming with infections of this flagellate. Also, frogs and toads harboring both *Opalina* and *Trichomonas* showed the number of these two parasites to be roughly in inverse proportions; those hosts harboring a heavy infection of *Opalina* showed a very light infection of *Trichomonas*, while hosts harboring light infections of *Opalina* carried heavy infections of *Trichomonas*. This numerical relationship was also observed in the case of rectal material from several specimens of *Hyla crucifer* and *Rana pipiens* tadpoles which were fed on a carbohydrate diet. Both species usually harbored a heavy infection of opalinids with few trichomonads; however, a carbohydrate diet usually promoted a heavy trichomonad infection in which case the opalinid infection disappeared. In individuals where the diet failed to produce heavy trichomonad infections the opalinid infection remained.

*Diplodiscus temperatus* IN RELATION TO *Opalina*

While studying the possible relationship of *Trichomonas* to opalinids in the green frog, it was observed that the trematode, *Diplodiscus temperatus*, was also usually present. After examination of many Ohio green frogs, several individuals of the same species were examined from Louisiana. In all of these frogs the fluke was found to be a common inhabitant. After continued observation of the green frog, and other amphibia as well, it was found that *Diplodiscus* and *Opalina* did not occur simultaneously in the same host. Furthermore, it was found that this fluke occurred in only a small percentage of green frog tadpoles, but that when present the opalinids were invariably absent. Experiments were therefore planned for a detailed study of the relations of these two parasites.

For these experiments tadpoles of the green frog were used. The tadpoles of this species are generally infected with opalinids; however, all the tadpoles from a certain small stream in Clinton County, Ohio, were found to be uniformly and entirely free from these ciliates. About 90 per cent of these tadpoles were infected with *Diplodiscus* with an average of about four flukes in each host. The smallest tadpoles, making up about 10 per cent of the collection, were free from flukes.

Approximately 50 tadpoles from this stream were fed cysts of *Opalina obtrigonoidea* Metcalf (1923), from the leopard frog, *Rana pipiens*. To infect these tadpoles, an entire rectum, containing active opalinids and cysts, was removed from an adult *R. pipiens* and placed in the vessel containing the experimental tadpoles, which fed freely upon this material. The adult opalinids if ingested died before reaching the rectum. Ingested cysts reached the rectum in about 5 hours where they were



observed either in the process of hatching or as excysted individuals measuring from 34 to 40 microns in length. Examination of flukes from these tadpoles showed their intestinal crura to be filled with these small opalinids (Fig. 1). When the flukes were placed in distilled water

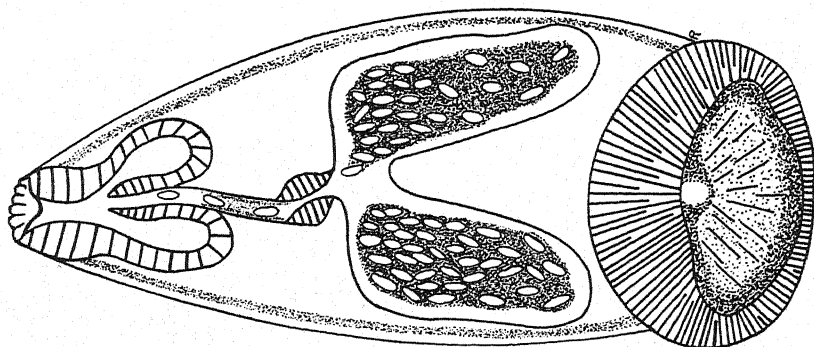


FIG. 1. Camera lucida drawing of *Diplodiscus temperatus*, details omitted. Opalinids, measuring 34 to 40 microns in length, are shown in the intestine having been ingested by the fluke soon after their excystment. Cilia are not shown.

their intestinal contents were regurgitated making possible a more accurate identification of these ciliates. Many of these protozoa in the lower part of the fluke's intestine were dead, other more recently ingested individuals near the esophagus were still moving about. In all cases the opalinids disappeared from the tadpoles in three days unless flukes were absent. As previously mentioned the smallest tadpoles did not harbor flukes and the opalinid infection of these was usually heavy and remained until they were killed and examined. In fact, the opalinids in these tadpoles grew to about 175 microns in length, dividing occasionally and forming cysts.

These experiments were repeated several times, and the observations on *Diplodiscus* confirmed on each occasion.

Experiments were next conducted to transfer flukes to the rectum of frogs harboring opalinid infections. Flukes were removed from both the adults and tadpoles of *Rana clamitans*, placed in Pütter's fluid and injected by means of a small pipette into the recta of a series of swamp cricket frogs, *Acris gryllus*, all of which contained opalinids. One fluke was transferred to each of 23 frogs which were opened 2 to 7 days after injection. In 10 specimens the fluke failed to establish itself and was not recovered, apparently having been carried out of the rectum with the fecal matter. Normal infections of opalinids remained in these 10 specimens. From 13 specimens the fluke was recovered and very few or no opalinids remained in the recta of these frogs. Four of the recovered flukes contained entire opalinids, and fragments of partly digested individuals.

Recently 8 tadpoles of *Rana clamitans* out of a series of 75 examined have offered additional evidence which confirms the observations on this relationship between the protozoa and the fluke. These 8 tadpoles showed simultaneous infections of flukes and opalinids. The opalinid infection in all cases was light while examination of the intestinal contents of the flukes showed the presence of opalinids in various stages of digestion. It is interpreted that these tadpoles had just recently become infected with the fluke and if the examinations had been made at a later period no opalinids would have been found. Incidentally, three specimens of *Nyctotherus cordiformis* were also present in one fluke and these still exhibited locomotion.

#### SUMMARY

On the basis of this study it is evident that *Diplodiscus* is partly responsible for the absence of opalinids from the adult green frog, *Rana clamitans*. Observations supporting this statement are as follows: (1) *Diplodiscus* ingests opalinids when they are present; (2) in localities studied by me the incidence of opalinid infection is very high in the green frog tadpoles, whereas the incidence of fluke infection is low; (3) conversely, the incidence of opalinid infection is practically nil in the adult green frogs, whereas the incidence of fluke infection is very high; (4) when flukes are transferred to the rectum of opalinid infected *Acris gryllus* by cloacal injection, the opalinids are reduced in number or completely disappear; (5) *Trichomonas* and *Opalina* in frogs and toads occur in roughly inverse proportions. A carbohydrate diet increases the number of trichomonads and decreases the number of opalinids.

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ACOLPENTERON URETEROECETES FISCHTHAL AND  
ALLISON, 1940, A MONOGENETIC TREMATODE  
FROM THE URETERS OF THE BLACK BASSES,  
WITH A REVISION OF THE FAMILY CAL-  
CEOSTOMATIDAE (GYRO-  
DACTYLOIDEA)\*

JACOB H. FISCHTHAL AND LEONARD N. ALLISON

*Acolpenteron ureteroecetes*, a monogenetic trematode belonging to the superfamily GYRODACTYLOIDEA, was first discovered by one of us (Fischthal) in the fall of 1939 in the ureters and urinary bladder of the large-mouthed bass, *Huro salmoides* (Lacépède). Since many parasites are common to both this species and the northern small-mouthed bass, *Micropterus dolomieu dolomieu* Lacépède, the latter were examined for this trematode and some were found to be infected.

This is the first record of a monogenetic trematode from the ureters or urinary bladder of fishes. Previously, such trematodes from the urinary bladders have been from frogs and turtles.

A preliminary description of this new genus and species has appeared in an abstract by Fischthal and Allison (1940).

Genus *Acolpenteron* Fischthal and Allison, 1940

*Generic diagnosis:* Monopisthocotylea. Head lappets, eyes and anchors absent. Haptor cup-like, possessing 14 marginal hooklets. Sensory hairs present, arising from papillae. Testis single, elongated. Ovary elliptical, median. Vagina ventral, near right margin of body. Intestinal crura joined posteriorly, without diverticula.

*Type species:* *Acolpenteron ureteroecetes* Fischthal and Allison, 1940.

*Acolpenteron ureteroecetes* Fischthal and Allison, 1940  
(Figs. 1-5)

*Specific diagnosis:* *Acolpenteron*. Adults,  $0.931 \times 0.105$  mm. Haptor,  $0.057 \times 0.096$  mm. Hooklets, 0.023 mm long. Testis elongated,  $0.053 \times 0.016$  mm. Seminal vesicle and coarse and hyaline prostates at left of, and opening into cirrus. Vas deferens sinistral, looping over prostates. Cirrus (0.045 mm long) with forked accessory piece; male opening mid-ventral. Ovary ( $0.062 \times 0.038$  mm) thick posteriorly, thin anteriorly. Oviduct, yolk ducts and seminal receptacle uniting anterior to ovary. Mehlis' gland large, extending from oötype to anterior third of testis. Uterus opening near cirrus. Vagina bent, opening ventral, near right margin posterior to cirrus. Seminal receptacle present. Vitellaria voluminous, extending from pharynx nearly to haptor. Pharynx muscular, 0.047 mm in diameter. Esophagus short. Intestinal crura without diverticula, confluent posteriorly.

Eggs ( $0.075 \times 0.056$  mm) operculate, anopercular process short, sharply bent and knobbed; 1 egg in uterus at a time; found free in ureters and urinary bladder of hosts; passed with urine in single-celled stage; hatching in water after 6-9 days. Larva ( $0.143 \times 0.020$  mm) ciliated, the cilia arising from 4 groups of cells at ends

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\* Contribution from the Department of Zoology, University of Michigan.

and middle of sides of body; 4 eyes present, their lenses facing laterally; anterior pair of eyes 0.006 mm in diameter, posterior pair 0.008×0.010 mm; 14 hooklets (0.013 mm long) on cup-like haptor (0.042×0.050 mm); pharynx muscular, 0.015 mm in diameter; head lappets and anchors absent; swimming path a long spiral, rotating to the right.

*Hosts:* *Huro salmoides* (Lacépède) and *Micropterus d. dolomieu* Lacépède.

*Locations:* Ureters and urinary bladder.

*Localities:* Huron River, Honey Creek, Whitmore Lake and West Lake in Washtenaw County, Michigan.

*Type and Paratypes:* U. S. Nat. Mus. Helm. Coll. No. 42096 and No. 44301.

Examination of 175 large- and small-mouthed basses varying in size from two inch fingerlings to 14 inch adults showed that approximately 36 per cent of these fish are infected with *A. ureteroecetes* (Table 1).

TABLE 1.—Incidence of infection and habitat preference of *Acolpenteron ureteroecetes*

<i>Huro salmoides</i> (Lacépède)—large-mouthed bass						
Locality	No. examined	No. inf.	% inf.	No. with worms in ureters only	No. with worms in bladder only	No. with worms in both places
Huron River ...	52	30	58	21	6	3
Saline River ...	5	0	0	0	0	0
Honey Creek ...	20	2	10	2	0	0
Whitmore Lake .	35	8	23	7	1	0
West Lake .....	3	3	100	3	0	0
Winan's Lake ..	3	0	0	0	0	0
Totals .....	118	43	36	33	7	3
				(77% of the 43 infected)	(16% of the 43 infected)	(7% of the 43 infected)
<i>Micropterus d. dolomieu</i> Lacépède—northern small-mouthed bass						
Huron River ...	42	15	36	11	2	2
Saline River ...	7	0	0	0	0	0
Whitmore Lake .	8	5	63	4	0	1
Totals .....	57	20	35	15	2	3
				(75% of the 20 infected)	(10% of the 20 infected)	(15% of the 20 infected)

The data also indicate that approximately 76 per cent of the infected black basses harbor worms only in the ureters, thus demonstrating a much greater predilection for the ureters as a habitat.

The black basses are usually infected with one to five worms. However, occasional heavy infections of 22, 39, and 52 adults, respectively, were found. In the latter case the worms were considerably crowded and several were found in the uriniferous tubules which empty directly into the ureters. No pathological conditions were observed.

The ureters and urinary bladders of fishes probably are more common habitats for monogenetic trematodes than is supposed by investigators in this field. We base this on the finding of *A. ureteroecetes* in these habitats in the black basses, and also on the more recent finding of another new species in the ureters of another species of fish.

## Family CALCEOSTOMATIDAE (Parona and Perugia, 1890)

Price (1937) emended the family characters of the CALCEOSTOMATIDAE (Parona and Perugia, 1890) as follows: "Cephalic gland ducts not concentrated into head organs but remaining scattered over a considerable area on either side of anterior end of body, the anterior end being expanded and forming head lappets. Haptor sucker-like but not strongly muscular, with or without large hooks, with or (?) without marginal hooklets. Intestine with short diverticula. Eyes present or (?) absent. Testis single. Cirrus simple, cuticularized. Vagina present or absent."

Genera recognized as belonging to this family are *Calceostoma* Beneden, 1852 and *Fridericianella* Brandes, 1894. It is the opinion of Dr. E. W. Price of the Bureau of Animal Industry and of ourselves that the genera *Acolpenteron*, *Anonchohaptor* Mueller, 1938 and *Anoplodiscus* Sonsino, 1890 should also be placed in this family (see discussion below). In order to accommodate these three genera, the family diagnosis must be further emended as follows:

Family CALCEOSTOMATIDAE (Parona and Perugia, 1890)  
Char. emend.

*Synonym*: Calceostomidae Parona and Perugia, 1890.

*Diagnosis*: Ducts of cephalic glands not concentrated into head organs but remaining scattered over a considerable area on either side of anterior end. Anterior end expanded and forming head lappets, or not expanded and head lappets absent. Haptor sucker-like but not strongly muscular, with or without large hooks, with or without marginal hooklets. Intestine with or without short diverticula. Eyes present or absent. Testis single. Cirrus simple, cuticularized. Vagina present or absent.

*Type genus*: *Calceostoma* Beneden, 1852.

## Key to Genera of CALCEOSTOMATIDAE

1. Pair of pseudosuckers present at anterior end of body. *Anoplodiscus* Sonsino
- Pseudosuckers absent at anterior end of body . . . . . 2.
2. Vagina absent . . . . . *Calceostoma* Beneden
- Vagina present . . . . . 3.
3. Head lappets absent . . . . . *Acolpenteron* Fischthal and Allison
- Head lappets present . . . . . 4.
4. Eyes absent; ovary tubular, median . . . . . *Fridericianella* Brandes
- Eyes present; ovary loops around right limb of intestine. *Anonchohaptor* Mueller

Genus *Calceostoma* Beneden, 1852

*Diagnosis*: Anterior end of body expanded and forming large curled head lappets. Haptor cup-shaped, armed or (?) unarmed. Intestinal limbs with numerous short diverticula. Eyes present. Testis elongated. Ovary branched. Vagina absent.

*Type species*: *Calceostoma calceostoma* (Wagener, 1857) Johnston and Tiegs, 1922.



In this genus Price (1937) included three species, *C. calceostoma* (Wagener, 1857) (syn., *C. elegans* Beneden, 1858), *C. inerme* Parona and Perugia, 1889, and *C. glandulosum* Johnston and Tiegs, 1922, and stated that no representative of the genus has been reported from North America.

Genus *Fridericianella* Brandes, 1894

*Diagnosis:* Head lappets not as prominent as in *Calceostoma*. Haptor cup-like, with one pair of small centrally placed hooks; marginal hooklets (?) absent. Eyes absent. Intestinal branches with lateral diverticula, united by commissure posterior to testis. Testis single, rounded. Ovary tubular, median. Vagina present, opening laterally near equator of body.

*Type species:* *Fridericianella ovicola* Brandes, 1894.

Brandes (1894) described the type and only species from specimens collected from the eggs of *Arius commersonii* Lac., a fresh- and brackish-water fish from South Brazil.

Genus *Anoplodiscus* Sonsino, 1890

*Diagnosis:* Anterior haptors in form of a pair of pseudosuckers situated at anterior end of body. Posterior haptor cup-like, unarmed. Eyes present. Intestine without diverticula, united by a commissure posterior to testis. Testis single, preequatorial. Ovary round, pretesticular. Vagina ventral, near right margin.

*Type species:* *Anoplodiscus richiardii* Sonsino, 1890.

Sonsino (1890) described the type species, *A. richiardii* from the gills of *Pagrus orphus* from the Mediterranean. Monticelli (1905) subsequently redescribed this species. The only other species so far included in this genus was described by Johnston (1930) as *A. australis* from the fins of *Sparus australis*, at Sydney Harbor, Australia.

Monticelli placed the genus *Anoplodiscus* in the ANISOCOTYLINAE Monticelli, 1903—a subfamily without a corresponding genus and consequently invalid—and later Tagliani (1912) erected for it the subfamily ANOPLODISCINAE, elevating Monticelli's invalid ANISOCOTYLINAE to the status of an invalid family ANISOCOTYLIDAE. Johnston and Tiegs (1922) considered *Anoplodiscus* as possibly belonging to the CALCEOSTOMATIDAE, subfamily DIONCHINAE, while Fuhrmann (1928) and Gallien (1937) placed it in the MONOCOTYLIDAE, subfamily PSEUDOCOTYLINAE. Price (1938) included "... *Anoplodiscus* in the Microbothriinae (family Microbothriidae, superfamily Capsaloidea), mainly because the general organization and the lack of haptor hooks suggest relationship with such genera as *Microbothrium*, *Leptocotyle* and *Leptobothrium*. On the other hand, the presence in *Anoplodiscus* of eyes and a cirrus with accessory piece suggests affinities with genera of the Gyrodactyloidea. It is possible that a restudy of the species of *Anoplodiscus* may show the haptor to be armed with minute hooks in which case it should be transferred to the family Calceostomatidae (Gyrodactyloidea)."

In a personal letter, Dr. E. W. Price makes the following comments:

"I have examined the specimens of the monogenetic fluke (*Acolpenteron ureteroecetes*) . . . and am of the opinion that they represent a new genus perhaps closely related to *Anoplodiscus*. In my paper (1938, J. Wash. Acad. Sc. 28: 187-188) I have discussed the systematic position of the genus *Anoplodiscus*. This genus was placed by me in the family Microbothriidae, subfamily Microbothriinae. . . . My present opinion is that *Anoplodiscus* and your new form (*Acolpenteron*) should be placed in the Calceostomatidae (Gyrodactyloidea)."

Genus *Anonchohaptor* Mueller, 1938

*Diagnosis:* Prominent head lappets present. Haptor disc-like without large hooks, but possessing 14 hooklets, 12 marginal and 2 central. Four eyes present. Intestine without diverticula, united by commissure posterior to testis. Testis single, small. Ovary looping around right limb of intestine. Vagina ventral, on right margin.

*Type species:* *Anonchohaptor anomalum* Mueller, 1938.

This genus contains only the type species which was described by Mueller (1938) from the gills of suckers from Chautauqua Lake, N. Y. Mueller (1938) records the following comments contained in a personal letter from Dr. E. W. Price: "This worm (appears to have) characters of both the superfamilies Gyrodactyloidea and Capsaloidea. In some respects it is quite like *Fridericianella*, if allowance be made for possible errors of observation by Brandes. The looping of the ovary around one of the intestinal limbs is more or less constant character of the Monocotylidae (Capsaloidea). The absence of large hooks (anchors) and bars is characteristic of *Fridericianella*, although eyes are supposed to be missing in the latter genus. The H-like formation of the gut is like both *Fridericianella* and *Anoplodiscus*. The nature of the copulatory organ is definitely of the type occurring in the Gyrodactyloidea (Dactylogyridae and Calceostomatidae) and not that of Capsaloidea. The anterior end of this form seems more capsaloid than gyrodactyloid. Personally, I am rather of the opinion that the form should be referred to the Calceostomatidae (Gyrodactyloidea)."

#### SUMMARY

1. *Acolpenteron ureteroecetes* originally described by Fischthal and Allison (1940) is described in further detail.
2. This is the first monogenetic trematode to be recorded from the ureters or urinary bladders of fishes.
3. Approximately 36 per cent of the large- and small-mouthed basses examined from Washtenaw Co., Michigan, were infected with this species; approximately 76 per cent of the infected fishes harbored this trematode in the ureters only.
4. It is our belief that the ureters and urinary bladders of fishes are more common habitats for monogenetic trematodes than is supposed by

investigators in this field. This is based not only on the finding of *A. ureteroecetes* in these habitats in the black basses but on the more recent finding of an additional new species in the ureters of another species of fish.

5. The family CALCEOSTOMATIDAE (Parona and Perugia, 1890) has been emended, and to it are now ascribed the genera *Acolpenteron* Fischthal and Allison, 1940, *Anonchohaptor* Mueller, 1938, and *Anoplodiscus* Sonsino, 1890, in addition to the previously included genera *Calceostoma* Beneden, 1852, and *Fridericianella* Brandes, 1894.

6. A key to the genera of the family CALCEOSTOMATIDAE is presented.

7. The diagnosis of each genus is followed by a list of species and a brief discussion.

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## EXPLANATION OF PLATE, p. 524

All figures refer to *Acolpenteron ureteroecetes*. The value of the scale is 0.05 mm for Figs. 1, 2 and 5, and 0.02 mm for Fig. 3.

## ABBREVIATIONS

AP	accessory piece	PG	pigment granules of disintegrated eyes
CG	cephalic glands	PH	pharynx
CIR	cirrus	SH	sensory hair
CP	coarse prostate	SR	seminal receptacle
E	esophagus	SV	seminal vesicle
H	haptor	T	testis
HP	hyaline prostate	UP	uterine pore
IC	intestinal ceca	UT	uterus
M	mouth	V	vagina
MG	Mehlis' gland	VD	vas deferens
MH	marginal hooklet	VDT	vitelline duct
OV	ovary	VIT	vitellaria
OVID	oviduct		

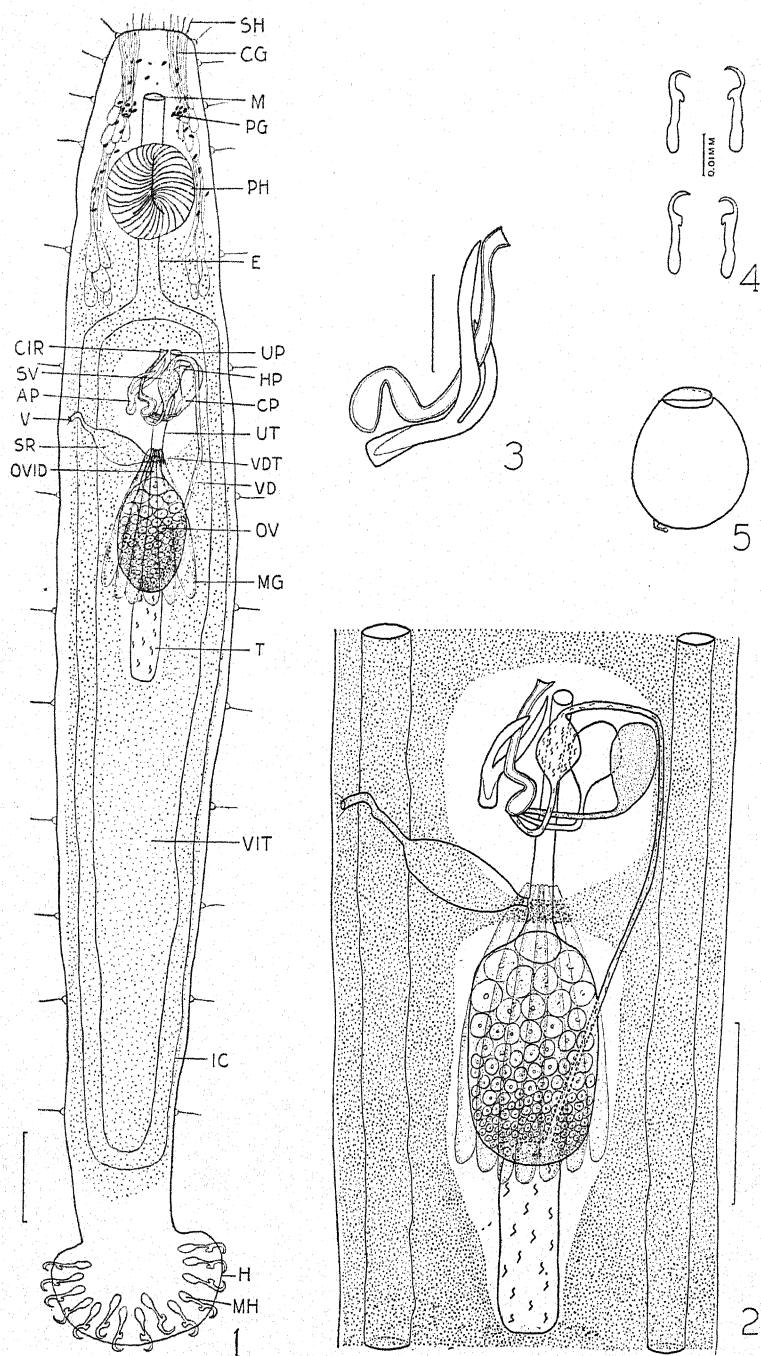
FIG. 1. Adult worm, ventral view.

FIG. 2. Reproductive complex, enlarged, ventral view.

FIG. 3. Cirrus and accessory piece.

FIG. 4. Marginal hooklets.

FIG. 5. Hatched egg showing operculum and posterior process.

*Acolpenteron ureteroecetes*



## STUDIES ON THE MORPHOLOGY AND BIOLOGY OF A PSILOSTOME FLUKE\*

SEYMOUR I. FELDMAN

During the late fall of 1939, approximately one hundred *Stagnicola reflexae* were brought into the laboratory supposedly infected with an echinostome cercaria. Careful study, however, showed that they were infected with a psilostome cercaria, closely related to the echinostome group, but lacking the collar of spines.

In checking the literature for the described species of closely related cercariae, the writer came upon one described by Cort (1914) as *Cercaria reflexae*, from *Lymnea reflexae* (= *Stagnicola reflexae*) from the vicinity of Chicago, Illinois. This cercaria resembled the one found at Edina, Minnesota, to a remarkable degree. However, since flame cell patterns were unknown at the time of this description (Cort, 1914), it was impossible to compare the two forms in this respect. Nevertheless, the writer is firmly convinced that they are identical.

Sewell (1922) placed *Cercaria reflexae* together with the cercaria of *Himasthla militaris* in the "Reflexae" group. The latter species is an echinostome which shows no spines in the cercarial stage, but has a very distinct collar of spines in the adult. Although Sewell recognized the close relationship between these two cercariae, in view of our present knowledge, he was incorrect in asserting that *Cercaria reflexae* was probably a larval stage of a species of the genus *Himasthla*.

### MATERIAL AND METHODS

The cercariae and rediae were best studied in the living condition. For the detailed morphology of the excretory system, cercariae were stained vitally with neutral red and Nile blue sulphate, and studied under a vaseline-ringed coverslip. Measurements of the cercariae were all taken from specimens fixed in the following manner: cercariae were set in a refrigerator (38° F) for 3 to 4 hours, after which time they were fixed in hot Gilson's fluid. This method was found to be very satisfactory for fixing cercariae in the extended condition. Fixed specimens were then stained with Mayer's paracarmine, dehydrated, cleared in methyl salicylate and mounted in clarite. Rediae were fixed in alcohol-acetic-formalin (A.F.A.), stained and mounted as described above.

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The adult trematodes secured in the course of the experimental work were studied in the living and fixed condition. They were fixed in A.F.A. and in hot Gilson's fluid, and stained with Semichon's acetic carmine, Ehrlich's acid haematoxylin and Mayer's paracarmine. The worms were then dehydrated through the alcohols, cleared in methyl salicylate and mounted in clarite. The adults were also fixed in Bouin's fluid, sectioned and stained with Ehrlich's haematoxylin-eosin or Delafield's haematoxylin-eosin.

#### EXPERIMENTAL RESULTS

All cercariae used in the experiments were obtained from naturally infected *Stagnicola reflexae*. The metacercariae of *Psilostomum reflexae* (the adult stage of *Cercaria reflexae*) were found naturally in *Stagnicola reflexae* and *Physa gyrina*. These two snails and *Heliosoma* sp. were experimentally infected with the cercariae in the laboratory. Many attempts were made to infect laboratory-reared tadpoles, but all were unsuccessful. Laboratory-reared snails of the species mentioned above were all infected in the following manner: two snails were placed in an isolation bottle with an infected *Stagnicola reflexae* producing large numbers of cercariae for 48 hours, after which time they were removed to another container. In each case the cercariae penetrated the snail and encysted in the mantle cavity; the metacercariae were readily seen macroscopically as white spheres against the black pigmented mantle cavity. They were infective five to seven days after encystment.

After feeding infective metacercariae to young chicks, mature worms were found in the small intestine, two to three inches in front of the caecum, five days after infection. One chick was autopsied eleven hours after infection and four young worms showing well-developed testes and the beginning of the development of the ovary and cirrus pouch were recovered.

Several species of birds and mammals were used in infection experiments with positive results only in chicks. Forty-seven chicks of several breeds were used in this series of infection experiments. The majority were infected soon after hatching and before being fed. Of the forty-seven chicks fed metacercariae, 18 or 38.3% were found parasitized at autopsy. From these 18 infected chicks, 59 worms or 3.2 worms per infected chick were obtained, although an average of 150 metacercariae were fed each chick. From these experiments, it became apparent that the chick was not a very satisfactory host for this trematode, since only 2% of the metacercariae fed developed.

Larson et al (1939), showed that the body temperature of mice could be raised from 100.6° F to 104.1° F if they were kept in a chamber at 98° F for three days. Reasoning along these lines, the writer attempted to approach avian temperatures in mice by this procedure, in order to de-

termine if a bird trematode might be made to mature in a mammal whose body temperature closely approximated that of a bird. Three mice were used in this experiment. Their body temperatures were raised by the procedure described above and they were then fed the metacercariae and maintained at 98° F. Three days later they were autopsied, and all mice were negative.

*Psilostomum reflexae*

The Cercaria

(Figs. 3, 4, 5)

*Description:* Relaxed, the body of the cercaria is elongate, sides more or less parallel with a slight tapering at the anterior end. Oral sucker powerful, terminal and slightly oval in outline 0.039–0.054 mm (mean 0.046 mm) long by 0.043–0.050 mm (mean 0.046 mm) wide. Acetabulum protrusible, considerably larger than oral sucker, 0.066–0.074 mm (mean 0.071 mm) in diameter, located just posterior to the mid body. Pharynx broadly oval, about one-half the diameter of the oral sucker; esophagus extends just anterior to the acetabulum where it bifurcates into the two intestinal caecae; the latter extend to the posterior end of the body. Ten to twelve cephalic duct openings present just anterior to the oral sucker.

Excretory bladder with one chamber; entire excretory system echinostome-like. Two large excretory siphons arise mesally with a common stem from the anterior end of the excretory bladder; siphons proceed laterally for a short distance and then anteriorly and dorsally in four loops to the posterior level of the acetabulum and continue again ventrally and anteriorly to the level of the pharynx in three or four short loops. The anterior part of each siphon contains many highly refractive granules. At the level of the pharynx the siphons make a wide loop and proceed posteriorly as the main excretory tubules to the posterior margin of the excretory bladder where they make another wide loop. The tubules proceed anteriorly to a short distance behind the acetabulum where they are no longer visible. The main collecting tubules are approximately one-half the diameter of the siphons. Twenty-eight flame-cell-like tufts or cilia were observed in the main collecting tubules. It was impossible to elucidate any flame cell pattern by any technique known to the writer because of the extensive distribution of the cystogenous glands. Forty-two pairs of flame cells and a median unpaired flame cell ventral to the posterior margin of the bladder were observed. Of these, nine pairs were found anterior to the fore margin of the acetabulum; thirty-three pairs were found scattered throughout the region posterior to the acetabulum. Tail 0.487–0.574 mm (mean 0.524 mm) by 0.052–0.070 (mean 0.061 mm) tapering posteriorly to a point; tail highly muscular. A narrow duct leads from the middle of the posterior margin of the excretory bladder into the tail region for a short distance where it bifurcates into a right and left fork at about one-third the tail length; no flame cells were observed in the tail. Tail provided with a dorsal and ventral fin fold which extends from the tip to the level of the excretory bifurcation.

The cercariae of *P. reflexae* were active swimmers, and under laboratory conditions remained motile for approximately twelve hours. Soon after, they settled to the bottom and crept along the surface of the isolation bottle in measuring-worm fashion with the aid of their powerful suckers for a short time. After fifteen hours most of the cercariae were dead. The cercariae emerged every morning between 6 and 10 AM, although in several instances they did not emerge until mid-afternoon. At these times it was observed that the intensity of light in the laboratory

was greatly reduced. Several experiments were attempted in order to determine if intensity of light had any significant effect on the time of emergence of the cercariae. In order to test this, one half of the infected snails were placed in a dark box and one half in front of a window. The snails left in the open were examined the following mornings and in all cases the cercariae had already emerged. The snails kept in the dark showed no swimming cercariae at the same time. Although this condition was observed on numerous occasions, available data do not justify a final conclusion regarding the effect of light intensity on the time of cercarial emergence.

#### The Redia

(Fig. 2)

*Description:* Body sacculate. Distinct collar with four lobes present in the anterior part of the body; birth pore located slightly posterior to one of the dorsal lobes. A pair of posterior locomotor appendages of variable size and activity are located at the posterior third of the body. Oral sucker poorly developed; large muscular pharynx situated just posterior to the oral sucker and terminal in position. Gut ventral, extending from the pharynx to the level of the locomotor appendages; filled with dark brown or orange brown granules. Germ balls and developing cercariae fill the entire body space of the rediae. Excretory system completely obscured by the developing cercariae.

The rediae of *P. reflexae* were studied from naturally infected *Stagnicola reflexae*. No mother rediae or sporocysts were observed. This was probably due to the fact that all the infected snails had infections of long duration. Daughter rediae were found in the liver, gonad, and kidney of the infected snail host in prodigious numbers, frequently destroying most of the tissue of these organs. When removed from the tissues, the rediae were of variable size; the older and larger were orange to brown in color, the younger and smaller forms colorless.

#### The Metacercaria

The metacercaria of *P. reflexae* differs from that of the closely related *P. ondatrae* Price, 1931 in the type of intermediate host utilized and in the thickness of the cyst wall. The former encysts in snails and has a three-layered cyst wall 0.039–0.042 mm wide, while the latter is characterized by a very thin cyst wall and is found in the lateral line canal and under the scales of several species of fish. The cysts of *P. reflexae* are spherical with a mean diameter of 0.259 mm while those of *P. ondatrae* are elongate and measure 0.32 mm by 0.21 mm. All attempts to excyst the metacercariae by rupturing the cyst wall or by means of artificial digestion methods were futile.

#### The Adult

(Fig. 1)

*Description: Psilostomum:* Body linguiform, bluntly rounded at either end. Length 1.46–1.83 mm (mean 1.47 mm); width remarkably uniform along most of

the body length, 0.42–0.62 mm (mean 0.46 mm). Entire surface beset with small triangular spines arranged in close alternate rows; spines become less numerous in the hind body. Two rows of cuticular spines in the region of the oral sucker distinctly separated from the rest of the body spines by a width of 0.02 mm; first of these two rows contains ten spines, second row twelve to fourteen. This latter condition is highly suggestive of the collar of spines found in the echinostomes. Oral sucker subterminal, 0.06–0.09 mm (mean 0.07 mm) in diameter. Acetabulum in anterior third of body, larger than oral sucker, 0.11–0.16 mm (mean 0.13 mm) in diameter. Prepharynx very short; pharynx oval, well developed, 0.05–0.07 mm (mean 0.06 mm) by 0.05–0.06 mm (mean 0.055 mm). Esophagus long, 0.20–0.23 mm (mean 0.22 mm), dividing to form the intestinal caecae just anterior to the acetabulum. Caecae increase in width as they extend toward the posterior part of the body. Excretory pore subterminal and ventral; bladder truncate; main tubules readily observable in the forebody of the living specimens as highly branched structures reaching the level of the pharynx.

Testes tandem, spherical or slightly oval, located in the middle of the hind body. Anterior testis 0.12–0.17 mm (mean 0.13 mm) by 0.11–0.20 mm (mean 0.18 mm); posterior testis 0.12–0.17 mm (mean 0.13 mm) by 0.14–0.21 mm (mean 0.19 mm). Cirrus pouch oval, slightly dorsal, either to the left or right of the acetabulum and never extending beyond its posterior margin. Seminal vesicle large, 0.13 mm in length, in some cases occupying most of the cirrus pouch; cirrus simple, slender and short. Prostate gland well developed. Genital pore situated between the acetabulum and the bifurcation of the intestine.

Ovary anterior to the testes, round or oval, slightly to the left of the median line of the body; diameter 0.09–0.11 mm (mean 0.10 mm). Shell gland present, well developed; yolk ducts pass to a voluminous yolk reservoir situated anterior to the anterior testis. Seminal receptacle and Laurer's canal not visible. Uterus short with few convolutions, containing six to eight eggs. Vitelline follicles large and closely distributed, extending to the level of the acetabulum on either side and converging posteriorly behind the hind testis to form a single compact mass filling most of the post-testicular region of the body. Eggs yellow-brown in color, 0.07–0.09 mm (mean 0.08 mm) by 0.05–0.06 mm (mean 0.056 mm).

*Habitat:* Small intestine.

*Host:* Domestic chicken (experimental).

*Collateral types:* U.S.N.M. No. 36762.

The above description of *P. reflexae* is based on a study of ten collateral types taken from the experimental chick host.

#### TAXONOMIC RELATIONSHIPS

The family PSILOSTOMIDAE was erected by Odhner in 1913 to include five genera of worms, all echinostome-like in their morphology and all parasites of birds. It was divided into two subfamilies, PSILOSTOMINAE Lühe, 1909 and ORCHIPEDINAE Skrjabin, 1913. The former subfamily, which includes the genera *Psilostomum* and *Psilochasmus*, is of chief interest here. The genus *Psilostomum* was erected by Looss in 1898 to include three species of worms. Of the original three, only the type species, *P. brevicolle* (= *platyurum* Mühling) still stands as valid.

Looss in his generic diagnosis, described the esophagus of *Psilostomum* as "fehlend oder sehr kurz." It was indeed unfortunate that he chose to describe this structure with so broad a term as "sehr kurz." In the eight known species of *Psilostomum*, the esophagus ranges from "no



esophagus" to one 0.324 mm in length (*P. arvicolae* Schulz and Dobrowa, 1933). *P. reflexae* falls somewhat between these two extremes, since its esophagus has a mean length of 0.22 mm. The problem of erecting a new genus for *P. arvicolae* and *P. reflexae* presents itself. After a careful study of the other morphological features, and after comparing these with similar characters in the recognized species of *Psilostomum*, it was not believed that there was any justification in erecting a new genus for this variation in morphology. Since the writer was unable to study any of the described species of *Psilostomum* with the exception of *P. ondatrae* Price, he has little choice but to accept them all as valid.

*P. reflexae* differs from *P. redactum* Nicoll, 1906, *P. varium* Linton, 1928 and *P. brevicolle* Creplin, 1829 by the presence of an esophagus. In the latter three species the esophagus is completely lacking. It also differs from *P. ondatrae* Price, *P. cygnei* Southwell and Kirschner, 1931 and *P. progeneticum* Wisniewski, 1932 by possessing an esophagus longer than 0.1 mm. *P. reflexae* differs from *P. arvicolae* Schulz and Dobrowa in that the latter lacks an oral sucker and has an acetabulum approximately 0.4 mm in diameter.

#### DISCUSSION

The life cycle herein described is the fourth known cycle of this PSILOSTOMIDAE. *P. reflexae* agrees morphologically and biologically with other psilostomes and echinostomes in their salient features. There are several specific differences in morphology mentioned in the text. *P. reflexae* is more host specific than any of its close relatives. *P. ondatrae* has been reported naturally from the muskrat, *Ondatra zibethica* and the California gull, *Larus californicus* by Price (1931) and from young chickens by Newsom and Stout (1933). It has also been reported from an osprey, *Pandion haliaetus carolinensis* and from Cooper's hawk, *Accipiter cooperi* by Beaver (1939) who also experimentally infected pigeons, chickens, ducks, and canaries. *Sphaeridiotrema globulus* has been found in a variety of ducks by Szidat (1937) and *Psilotrema spiculigerum* has been found in ducks, turtle doves, hens, quails, and mice by Mathias (1925).

The writer attempted to infect ducks, sparrows, pigeons, rats, guinea pigs, mice, rabbits, cats, and chicks. Only in the last mentioned hosts were the adults of *P. reflexae* recovered.

The echinostomes in general, are the least host specific of the trematodes known to the writer. This lack of host specificity is attested by the world wide distribution of these forms. From the small amount of work done with the psilostomes, it appears that they are more host specific than are the closely related echinostomes. As our knowledge of the trematode fauna of the world increases, we will be able to solve more of

problems related to host specificity. At present, the nature of host city in the TREMATODA is to a very large extent a matter of conjecture.

# SUMMARY

1. *Ceraria reflexae* Cort, 1914 was found in *Stagnicola reflexae* in the vicinity of Edina, Minnesota.

2. Rediae were found naturally in the same snail host. None of the asexual stages in the primary snail host were produced experimentally.

3. Metacercariae were found naturally in the mantle cavity of *Physa* and *Stagnicola reflexae*. These two snails and *Heliosoma* sp. have been experimentally infected.

4. Metacercariae were found in the same snail which produced the cercariae. The former are infective five to seven days after encystment.

5. *Psilostomum reflexae* (Cort, 1914) was found in the small intestine of the chick after feeding the metacercariae. The adults mature in six days. No pathological anatomy was observed.

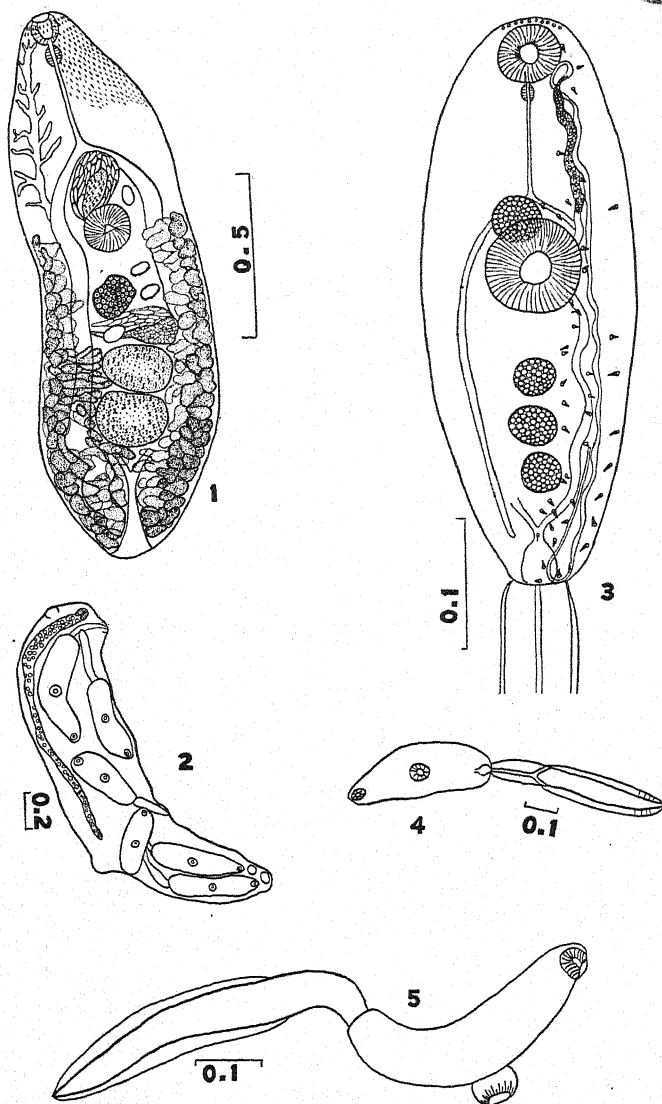
6. The natural host of this trematode is probably another snail eating bird, but as yet no natural infections have been found.

7. Eggs pass out with the feces in an early segmentation stage. Development occurs on the outside. Miracidia are not known.

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- FIG. 1. Adult *Psilostomum reflexae* showing general morphology.  
 FIG. 2. Redia of *P. reflexae*.  
 FIG. 3. Cercaria of *P. reflexae* showing details of the excretory system.  
 FIG. 4. Cercaria showing excretory system of the tail region and the caudal fin folds.  
 FIG. 5. Cercaria showing protrusible acetabulum.

All scales in millimeters





THE "GOPHER," *CITELLUS RICHARDSONII* (SABINE),  
AS AN EXPERIMENTAL HOST FOR  
*TRICHINELLA SPIRALIS*

EDWARD P. OFFUTT, JR., AND O. R. MCCOY\*

*Trichinella spiralis* is noted for its ability to parasitize a wide variety of hosts. Experimentally, it is able to develop in all species of mammals that have been tested and even in certain birds (1, 2). Although the course of infection has been studied quantitatively in only a few animals, it is apparent that the parasite exhibits different degrees of pathogenicity in different hosts. Thus, rats are relatively insusceptible and readily develop a high degree of resistance to reinfection (3, 4), whereas guinea pigs (5, 6, 7) and monkeys (8), are relatively susceptible and are killed by comparatively small doses of larvae. Compared to rats, these animals develop only a slight degree of resistance to reinfection.

The purpose of the present experiments was to test the susceptibility of the "gopher," *Citellus richardsonii* (Sabine), to infection by *Trichinella spiralis* and to measure the ability of this host to develop resistance to reinfection.

MATERIALS AND METHODS

The experimental host, *Citellus richardsonii* (Sabine), commonly called the "gopher," is a small rodent which is widely distributed in the North Central States and the adjacent parts of Canada. The adult animals vary in weight from 400 to 1000 gm. They live in burrows in pastures and fields. They are usually herbivorous, but may be sufficiently carnivorous to devour sick or weakened members of their own or related species. As far as the writers are aware, *Citellus richardsonii* (Sabine) has never been reported as a natural host for *Trichinella spiralis*.

The animals used in the present experiments were captured in North Dakota. In the laboratory they were confined in metal cages and fed a diet of commercial dog chow. They were infected by the administration through a stomach tube of *Trichinella* larvae that had been obtained by digestion from the muscles of infected rats. The methods used for counting the adult worms and the encysted larvae in the infected animals were similar to those used in earlier experiments with rats (3). (The counts of adult worms include the worms found in the cecum and large intestine as well as those present in the small intestine. Comparatively few worms, usually less than 10 per cent, were found in the former location.)

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## EXPERIMENTAL

Twenty-three "gophers" were infected with from 2 to 40 *Trichinella* larvae per gm of body weight (Table 1). Five of them (No. 1, 2, 4, 17,

TABLE 1.—Course of infection in "gophers" that were fed from 2 to 40 *Trichinella spiralis* larvae per gram of body weight

"Gopher" No.	Larvae per gm of body weight	No. of larvae fed	Died — days after infection	No. of adult worms in intestine	Per cent of larvae fed	No. of larvae in muscles	Ratio to infecting dose
<i>Experiment 1</i>							
1	40	24,400	4	12,860	53	.....	...
2	40	12,400	16	2,720	22	.....	...
3	40	17,200	31	2,960	17	2,810,000	163
4	20	12,800	5	7,200	56	.....	...
5	20	13,600	31	1,340	10	1,670,000	123
6	20	12,800	72*	0	0	2,313,000	181
7	10	4,800	77	210	4	1,778,000	370
8	10	5,800	80	.....†	..	1,815,000	313
9	10	6,200	79	.....†	..	1,671,000	270
10	5	2,550	98*	.....†	..	978,000	383
11	5	2,600	105*	.....†	..	898,000	345
12	5	3,300	91	40	1	1,418,000	430
13	2	1,100	58	240	22	1,028,000	935
14	2	960	98*	.....†	..	233,000	243
15	2	1,000	105*	.....†	..	937,000	937
16	2	980	95	.....†	..	620,000	632
<i>Experiment 2</i>							
17	5	3,150	14	1,720	54	.....	...
18	5	3,650	23	2,440	67	.....	...
19	5	3,680	37	2,170	59	1,900,000	521
20	5	2,150	39	2,150	51	1,613,000	750
21	5	4,300	56	870	20	1,678,000	390
22	2	1,020	31	340	33	460,000	451
23	2	1,020	70	0	0	438,000	430

\* Animals that were killed.

† Reinfected before death.

and 18) died within 4 weeks, before it was possible to make an accurate count of the number of larvae in the muscles. Seven other animals were reinfected in order to test their resistance, thus interfering with the determination of the number of adult worms present in the intestine as a result of the first dose of larvae. The data for the remaining 11 animals are complete.

Each of the 23 "gophers" used became infected with the parasite and each, with only one or two exceptions, showed a marked loss of weight during the course of the experiments. Substantial numbers of adult worms were found in the intestines of nearly all of the animals that died or were killed at a time more than 4 weeks after infection. Thus, two "gophers" (No. 13 and 21) that died after 8 weeks harbored 240 and 870 worms, respectively, representing 22 and 20 per cent of the numbers of larvae fed. In two instances (No. 12 and 16), a few worms still persisted 13 weeks after the feeding of the larvae. As far as the writers are aware, this is the maximal length of life that has been reported for adult trichinae in any host.

A measure of the susceptibility of a host to *Trichinella* infection is provided by the ratio of the number of the larvae that encyst in the muscles to the number of the larvae that were originally fed. In 18 of the "gophers," this ratio showed great variation, the range being from 123 to 937 times the infecting dose and the average 437 times. Part of this variation may be explained by the fact that many of the animals died while invasion of the muscles was still taking place, thus permitting the young larvae that had not yet encysted to be lost during the process of digestion.

Five "gophers," which survived for 13 weeks after the initial dose of larvae, were fed a second dose of from 2 to 5 larvae per gm of body weight. One animal died and the others were killed within 2 weeks after the second infection. All of them showed numerous adult worms in their intestines, representing from 40 to 100 per cent of the larvae fed in the second dose. Two other "gophers" (No. 8 and 9) acquired a second dose of larvae accidentally by feeding upon the carcass of an infected "gopher" that had died in their cage. Both animals died within 3 days and were found to harbor 15,400 and 11,530 adult worms, respectively. These few tests discouraged further reinfection experiments, because it seemed evident that any immunity that could be demonstrated in "gophers" would be insignificant as compared to the high degree of immunity developed by rats. It is possible, however, that some resistance to reinfection might be demonstrated in "gophers" if smaller test doses were fed to animals that had had a longer time in which to recover from the initial infection.

#### DISCUSSION

From the data available, as Roth (6) has pointed out, guinea pigs, monkeys and humans appear to be the most susceptible hosts to *Trichinella* infection, whereas rats, mice, rabbits and swine are relatively insusceptible. The present experiments indicate that the "gopher," *Citellus richardsonii* (Sabine), is also a host that is very susceptible to trichiniasis. Ten of 14 animals died following infection with doses as small as from 2 to 5 larvae per gm of body weight. Adult worms, furthermore, persisted in the intestines of the "gophers" for longer periods of time than have been reported for other hosts, the maximal period observed being 13 weeks. The ratio of the number of the larvae encysted in the muscles to the number of the larvae fed averaged 437 in 18 "gophers." This figure is much lower than that for guinea pigs (approximately 1,000 (6)) or for monkeys (700 (8)), but it is higher than the ratio for rats (100 to 200, depending upon the size of the initial infection (9)). The low ratio in the "gophers," as compared to the ratios found in guinea pigs and monkeys, may be explained by the fact that on the average only about 50 or 60 per cent of the larvae fed to the "gophers" became established in the intestine as adult

worms, whereas this percentage is usually greater in the other two hosts mentioned.

## SUMMARY

The "gopher," *Citellus richardsonii* (Sabine), was found to be very susceptible to infection with *Trichinella spiralis*. Ten of 14 animals fed doses as small as from 2 to 5 larvae per gm of body weight succumbed. Adult worms persisted in the intestines of most animals for long periods of time, 13 weeks being the maximal period observed. Five "gophers" that were reinfected showed no resistance to the test doses of larvae that were employed.

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## RESEARCH NOTES

### A CASE OF CORRELATION BETWEEN INFECTION OF SNAIL HOSTS WITH *CRYPTOCOTYLE LINGUA* AND THE HABITS OF GULLS

During the summer of 1939, the writer, while at the Isles of Shoals Marine Biological Laboratory, had the opportunity of examining a large number of *Littorina littorea* for the immature stages of trematodes. The snails were secured from Duck Island, Appledore Island, and Smuttynose Island of the Isles of Shoals group off Portsmouth, N. H., and from Boone Island, Maine. In all, 817 snails of the species *Littorina littorea* were examined. Of this group, 75 were infected with immature stages of *Cryptocotyle lingua*, a common trematode of gulls.

A tabulation of the results of the examination of these snails shows that there is a correlation between the percentages of infected snails from various areas and the relationship of the gulls to these same areas. In areas where there are docks from which the gulls are continuously frightened, the writer found no *L. littorea* infected with *C. lingua*. On the other hand, in the feeding areas of gulls, six collections involving a total of 501 snails showed 6.2% of the snails infected. In the individual collections of this group, the percentages ran from 3.2% to 8.4%. The highest incidence of *C. lingua* in *L. littorea* is found near roosting and nesting sites of the gulls. Of the 86 snails examined from roosting areas, 17 or 19.8% were infected. A slightly higher incidence of infection, 20.9%, was found in the 134 snails examined from two nesting sites. In one nesting area, where the colony is small and somewhat scattered, the incidence ran only 14.3% while 30.0% of the snails from Duck Island were infected. Here the colony is large and crowded.

The localization of areas showing different percentages of infection of *C. lingua* in the snail host is in agreement with Stunkard's assertion (1930. J. Morphol. and Physiol. 50: 143-191) that there are in this species no free-living miracidia but that the eggs containing mature larvae are ingested by snails and the miracidia escape within the intestine of the snail. If there were free-living miracidia, one would not expect such a distinct difference in rate of infection between areas in many instances only a few hundred yards apart. The writer found that when fecal material from infected gulls was squirted against sea-lettuce or other marine vegetation, the eggs often adhered to such material. *L. littorea* ingested large numbers of the eggs when kept in aquaria with vegetation contaminated with fecal material from infected gulls. Thus it is probable that eggs contained in gull feces adhere either to the rocks or to the vegetation and that many of these eggs are eventually ingested by the snails. Such a contention would account for the fact that the miracidia apparently are not washed about by the waves. If they were carried about by the waves and currents or were capable of locomotion, one would expect a rather uniform distribution of the infection in snails over a large area.—C. CLAYTON HOFF, Urbana, Illinois. Contribution from the Zoological Laboratory of the University of Illinois, No. 588.

### THE HELMINTH FAUNA OF A RACCOON

In August, 1940, on the Edmund Niles Huyck Preserve, Rensselaerville, Albany County, New York, a male raccoon, *Procyon lotor lotor* (Linnaeus), of approximately 12 pounds weight was trapped, and its alimentary and circulatory systems examined for parasites. The locations from which the parasites were taken in the alimentary canal were noted to see whether quantitative data could be gathered to show that certain regions of the digestive tract were preferred over others.

Two species of helminths, a nematode, probably *Physaloptera maxillaris*, and a tapeworm, *Mesocostoides* sp., were removed from the alimentary canal. The distribution of the former was as follows: 29 individuals in the region of the greater curvature of the stomach, 11 individuals 6 inches down the small intestine from the



pyloric sphincter, and one individual 12 inches down the intestine from the pyloric sphincter. The preference for the stomach bears out the statement of Hall (1916, Proc. U. S. Nat. Mus. 50: 212) concerning the region of the alimentary canal preferred by members of the genus *Physaloptera*: "Parasitic in the digestive canal, especially the stomach, of mammals, birds, and reptiles."

Sixteen individuals of *Physaloptera* were removed from the postcaval vessel of the circulatory system. The presence of these individuals in the circulatory system is apparently abnormal, since members of this genus are typically parasitic in the alimentary canals of reptiles, birds, and mammals.

Fifteen individuals of the genus *Mesocestoides* were distributed in the small intestine in a zone from 15 to 20 inches above the point of union of the small intestine with the large intestine.

Acknowledgment is made to Dr. Benjamin Schwartz, Washington, D. C., for identifying the two helminths.—WILLIAM MARCUS INGRAM, *Zoological Laboratory, Cornell University*.

#### HEMATOZOA FROM CALIFORNIA BIRDS

In the course of routine examination of a series of 55 birds collected about the Los Angeles area blood smears and impression preparations of internal organs were studied. In addition smears of the peripheral blood of 75 birds caught for banding purposes on the campus of the University of California at Los Angeles were examined. The following summary is a record of the types of parasites and the numbers of infected birds found in the course of this survey:

##### *Haemoproteus*

Brown towhee (*Pipilo crissalis senicula*) (12), California jay (*Aphelocoma californica californica*) (7), western mourning dove (*Zenaidura macroura gambeli*) (3), spotted towhee (*Pipilo maculatus megalonyx*) (2), Gambel sparrow (*Zonotrichia leucophrys gambeli*) (2), California thrasher (*Toxostoma redivivum*) (1), California quail (*Lophortyx californica vallicola*) (1), Cooper hawk (*Accipiter cooperi*) (1).

##### *Plasmodium*

California thrasher (3), California jay (3), western mockingbird (*Mimus polyglottos leucopterus*) (1).

##### *Leucocytozoon*

California jay (4), California thrasher (2), brown towhee (1), screech owl (*Otus asio*) (1).

##### TRYPANOSOME

Brown towhee (2), California jay (1), long-tailed chat (*Icteria virens longicauda*) (1).

##### MICROFILARIA

Brown towhee (7), California thrasher (5), California jay (3), white-rumped shrike (*Lanius ludovicianus excubitorides*) (1), pallid wren-tit (*Chamaea fasciata henshawii*) (1), ash-throated flycatcher (*Myiarchus cinerascens cinerascens*) (1), screech owl (1).

One pallid wren-tit was infected with a parasite of undetermined nature which was found only within the monocytes. This parasite, which resembled closely the haemogregarines of lower forms, seemed to be particularly abundant in the heart blood.

The following birds were examined but recorded as negative: California quail (28), brown towhee (13), pallid wren-tit (5), spotted towhee (4), California thrasher (4), western mourning dove (2), Gambel sparrow (2), western tanager (*Piranga ludoviciana*) (2), cactus wren (*Heledoytes brunneicapillus couesi*) (2), white-rumped shrike (2), California jay (2), hermit thrush (*Hylocichla guttata*) (2), song sparrow (*Melospiza melodia*) (2), western mockingbird (2), bush-tit

(*Psaltiriparus minimus minimus*) (1), phainopepla (*Phainopepla nitens*) (1), horned-lark (*Otocoris alpestris*) (1), golden-crown sparrow (*Zonotrichia coronata*) (1).

There was a much higher infection (60 per cent) found in those birds in which complete examination was made, as compared with the infection (18 per cent) found in those birds in which only peripheral blood was examined. Similarly, 17 infections with more than one parasite were recorded for those birds which were completely examined, whereas no cases of multiple infection were recorded from those birds from which only peripheral smears were examined.

From this survey the following are considered new host records:

*Haemoproteus*: Brown towhee, spotted towhee, Cooper hawk, Gambel sparrow, California thrasher.

*Plasmodium*: California thrasher, western mockingbird, California jay.

*Trypanosome*: Brown towhee, long-tailed chat, California jay.

J. FREDERICK WOHNUS AND DWIGHT L. RYERSON, *Department of Zoology, University of California at Los Angeles.*

#### PARAGONIMUS IN A CAT FROM NORTH CAROLINA

Two specimens of *Paragonimus kellicotti* have been found in a cat from eastern North Carolina. Both were adults, one lying free in the thoracic cavity, and the other embedded in the lung tissue. No young specimens could be found, either in the lungs or in other parts of the body. As far as is known to the writer this is the first time *Paragonimus* has been reported from this state, although Smith (1911, Proc. Path. Soc. Phila., N. S. 14: 64) has found the adults in a wild cat from South Carolina. Metacercariae have been found in crayfishes from Virginia and West Virginia by La Rue and Ameel (1937, J. Parasitol. 23: 282).—A. B. HARDCASTLE, *Department of Zoology, Duke University, Durham, North Carolina.*

#### THE POLYCLAD, *HOPLOPLANA INQUILINA THAISANA* PEARSE, 1938, FROM THE MANTLE CAVITY OF OYSTER DRILLS

In May, 1938, a small turbellarian was detected in the mantle cavity of the oyster drill, *Urosalpinx cinerea* Say. Dr. Libbie Hyman has kindly identified this form as *Hoploplana inquilina thaisana* Pearse, 1938. Similar worms were later obtained from the rarer gastropod, *Eupleura caudata* Say. Specimens have been obtained from oyster drills from a number of areas within the oyster-growing regions of Delaware Bay.

In studying the distribution and incidence of this worm the oyster drills are brought to the laboratory where they are crushed and the soft parts removed and placed in beakers of sea water in lots of 30–50. The turbellarians soon leave the drill meats and climb the sides of the beaker from whence they are taken for isolation and study. This method has objections in that all worms may not climb up the sides and because multiple infections are missed. Since the latter are relatively few in number this is not a serious omission, and if the data are used only for determining relative incidence the former objection is also minimized. Using the above method 7,886 *Urosalpinx* were crushed in the past 3 years yielding 266 *Hoploplana* or 3.4 for every 100 drills crushed. From 494 crushed *Eupleura* only 6 specimens or 1.2 per 100 were obtained. A number of the *Urosalpinx* (640) were examined individually immediately after crushing by opening the mantle cavity and searching for worms, and 28 were found to be infected. Twenty-four of these (89 per cent) had only one *Hoploplana* in the mantle cavity, 3 had 2 worms and only 1 had 3 worms. There were 33 lots of drills crushed with worm incidence varying from none in a lot of 400 drills to 18 in a lot of 60 drills. Likewise the incidence varied from 0 in some parts of the bay to 10 worms per hundred drills crushed in drills collected from other areas. Heaviest incidence occurred in drills taken from a station along the Cape May Shore of Delaware Bay in the tidal zone. Drills from deeper waters seem to be either lightly attacked or to have no worms at all.

The turbellaria are oval and flat ranging in size in the living from  $0.6 \times 0.3$  mm to  $5.0 \times 3.0$  mm, most specimens being from 1-3 mm long and about three-fourths as wide. There are conical tentacles with clusters of tentacular eye spots in and around the bases of the tentacles and cerebral eye spots in 2 clusters more anteriorly. The number of tentacular eye spots varies significantly with the size of the turbellarian, totalling only 7 in a worm  $0.8 \times 0.6$  mm. There were 34 in a worm  $3.1 \times 2.7$  mm. The penis has a small pointed stylet and the uteri are not united anterior to the pharynx. Uteri distended with ova were seen in the larger specimens in the late spring and summer of 1939 and 1940. In June and July, 1939, and September, 1940, oviposition occurred when the turbellaria were isolated in watch glasses containing sea water, the egg masses being laid in a single or occasionally double coiled string. In 4 batches counted there were 144, 342, 245 and 50 eggs. The ova in one batch measured  $74-81 \mu$  and in another  $89-95 \mu$  in diameter.

The salinity of the water from which the *Urosalpinx* were obtained usually ranges from 18-25 parts per mille. When live and uninjured oyster drills were being experimentally studied for death rate and survival in test waters of low salinity (6-11 p.p.m.) it was noted that the *Hoploplana* infecting them would abandon the host under these unfavorable conditions and could be found on the glass walls of the container. This phenomenon occurred in salinities of 6, 7, 8, 9, 10 and 11 p.p.m. There is evidence that the turbellarians were injured by these conditions and frequently appeared moribund when discovered.

The genus *Hoploplana* now has representatives in the Eastern United States in *Busycon* from the Woods Hole area (1894, Wheeler, J. Morph. 9: 195-201) in *Thais* taken from Apalachicola Bay, Florida (1938, Pearse, Proc. U. S. Nat. Mus. 86: 67-98) and in *Urosalpinx* and *Eupleura* from Delaware Bay reported here. The New Jersey and Florida specimens agree in size and are both smaller than the Woods Hole specimens.

*Busycon* from Delaware Bay uniformly have been found free from this genus of polyclad.

Much of interest would arise from studies of these molluscs and their mantle cavity commensals throughout their geographic range.

Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project #65-1-22-477.—LESLIE A. STAUBER, Oyster Research Laboratory, N. J. Agricultural Experiment Station, Bivalve, New Jersey.

#### NEW NAMES FOR *METABRONEMA SALVELINI* FUJITA AND *CYSTIDICOLA MINUTA* FUJITA

Dr. Benjamin Schwartz of the United States Bureau of Animal Industry has kindly called my attention to the fact that *Metabronema salvelini* Fujita (1939, J. Fac. Agric. Hokkaido Imp. Univ. 42: 239-266) was preoccupied by *M. salvelini* (Fujita, 1922) Baylis (1935, Ann. and Mag. Nat. Hist. 10 s. 16: 378-380) and *Cystidicola minuta* Fujita (1940, Jap. J. Zool. 8: 377-394) by *C. minuta* Rodhain and Vuylsteke (1934, Rev. Zool. et Botan. Africaines 24: 406-409). Accordingly, I wish to propose for these homonyms the new names *Metabronema ishiiii* and *Cystidicola chika* for *M. salvelini* Fujita and *C. minuta* Fujita, respectively.—TSUNENOBU FUJITA, Faculty of Agriculture, Hokkaido Imperial University, Sapporo, Japan.

#### GENUS NAME *MARTINEZIA* CHANGED TO *MARTINEZIELLA*

We have been informed by Dr. Enrique Beltran that the genus name *Martinezia*, which we proposed for a species of ameba discovered in the intestine of an iguana, *Ctenosauria acanthura* (J. Parasitol., 1940, 26: 319-321), has already been used for an orthopteron. We now propose the genus name *Martinezziella* in place of *Martinezia*.—ROBERT HEGNER and REDGINAL HEWITT, Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F.

# INDEX FOR VOLUME 27, NOS. 1-6

(Index for December Supplement, 1941, begins page 553)

<i>Acolpenteron ureteroecetes</i> , monogenetic trematode from ureters of black basses, with revision of family Calceostomatidae .....	517
ALLISON LEONARD M. (see Fischthal and Allison) .....	517
AMERICAN SOCIETY OF PARASITOLOGISTS:	
Announcement of place of 17th annual meeting .....	282
Changes in membership roster .....	278
Minutes of 16th annual meeting, Philadelphia, Pa., Dec. 31, 1940 .....	276
Preliminary announcement, 17th annual meeting, Dallas, Texas, Dec. 29-31, 1941 .....	472
Program and abstracts, 17th annual meeting, Dec. 29-31, 1941, Dallas, Texas .....	December, 1941, Supplement
Report of the Committee on Nomenclature concerning <i>Trichuris</i> vs. <i>Trichocephalus</i> , and the status of <i>Diocetophyma</i> , <i>Diocetophyme</i> and <i>Eustrongylus</i> .....	279
Amoebae, <i>E. histolytica</i> -like, found in monkeys, morphology of .....	327
Amphibian nematodes from Gaspé peninsula .....	59
Anoplocephaline cestodes of hares and rabbits, studies on life history of .....	299
Anthelmintic studies on mono ethers of dihydroxy benzenes .....	357
Avian toxoplasmosis with invasion of erythrocytes .....	245
<i>Balantidium coli</i> and pinworm in a chimpanzee .....	366
BARTON, G. D. (see Pratt and Barton) .....	283
BEAVER, PAUL C. Studies on the life history of <i>Euparyphium melis</i> (Trema- toda: Echinostomidae) .....	35
BEAVER, PAUL C. The life history of <i>Echinocasmus donaldsoni</i> n. sp., a trem- atode (Echinostomidae) from the pied-billed grebe .....	347
Benzenes, dihydroxy, anthelmintic studies on mono ethers of .....	357
Birds, blood parasites of, of District of Columbia and Patuxent Research Refuge vicinity .....	379
Birds, California, hematozoa from .....	540
BLACKLOCK, D. B. AND T. SOUTHWELL. A guide to human parasitology for medical practitioners (Review) .....	274
Blood parasites of birds of the District of Columbia and Patuxent Research Refuge vicinity .....	379
Bluegill, a new dactylogyrid genus from .....	159
Bonito, <i>Sarda sarda</i> , two new trematodes from .....	183
<i>Boophilus annulatus microplus</i> , cattle tick, examinations of wild animals for, in Florida .....	465
BRACKETT, STERLING (see Cort, Olivier and Brackett) .....	437
<i>Branchellion ravenelii</i> , leech, rediscovery and morphology of .....	289
Bullhead, <i>Ameiurus nebulosus</i> , a new cestode from .....	221
Calceostomatidae (Gyrodactyloidea), revision of the family .....	517
Cat, <i>Paragonimus</i> in a .....	541
Cattle in Alabama, oöcysts of coccidia from .....	203
Cattle tick, <i>Boophilus annulatus microplus</i> , examinations of wild animals for, in Florida .....	465
<i>Cercaria douglasi</i> and its relation to cercaria of <i>Cotylurus flabelliformis</i> .....	343
<i>Cercaria elongata</i> from a new snail host in Minnesota .....	187
Cercaria, stylet, partial twinning in .....	92
Cestode, <i>Corallobothrium parvum</i> n. sp. from common bullhead .....	221
Cestode, <i>Gryporhynchus tetrorchis</i> , a new dilepidid from the great blue heron .....	171
Cestode, variation in <i>Raillietina</i> ( <i>Skrjabinia</i> ) <i>variabilis</i> from prairie chicken in Illinois .....	97

Cestode, anoplocephaline, of hares and rabbits, studies on life history of .....	299
CHANDLER, ASA C. A case of urinary myiasis .....	465
CHANDLER, ASA C. A new spiruroid nematode, <i>Habronema americanum</i> , from the broad-winged hawk, <i>Buteo platypterus</i> .....	184
CHANDLER, ASA C. Helminths of muskrats in southeast Texas .....	175
CHANDLER, ASA C. The specific status of <i>Moniliformis</i> (Acanthocephala) of Texas rats, and a review of the species of this genus in the western hemisphere .....	241
CHANDLER, ASA C. Two new trematodes from the bonito, <i>Sarda sarda</i> , in the Gulf of Mexico .....	183
CHEATUM, E. L. <i>Dendritobilharzia anatinarum</i> n. sp., a blood fluke from the mallard .....	165
Chicken, prairie, variation in a new species of cestode from .....	97
Chickens and robins, studies on gapeworm in .....	369
Chimpanzee, <i>Balantidium coli</i> and pinworm in .....	366
CHITWOOD, B. G. AND M. B. CHITWOOD. An introduction to nematology (Review) .....	273
CHRISTENSEN, JOHN F. The oöcysts of coccidia from domestic cattle in Alabama (U. S. A.), with descriptions of two new species .....	203
Coccidia in partridges and pheasants in Denmark .....	29
Coccidia, oöcysts from domestic cattle in Alabama .....	203
<i>Contracaecum multipapillatum</i> , redescription of .....	505
<i>Cooperia curticei</i> , spicule length in, as a measure of favorable intestinal environment for this intestinal nematode of sheep .....	449
CORT, W. W. AND LOUIS OLIVIER. Early developmental stages of strigeid trematodes in the first intermediate host .....	493
CORT, W. W., LOUIS OLIVIER AND STERLING BRACKETT. The relation of physid and planorbid snails to the life cycle of the strigeid trematode, <i>Cotylurus flabelliformis</i> (Faust, 1917) .....	437
CORT, W. W., LOUIS OLIVIER AND D. B. McMULLEN. Larval trematode infection in juveniles and adults of <i>Physa parkeri</i> Currier .....	123
CORT, W. W. (see Olivier and Cort) .....	343
<i>Cotylurus flabelliformis</i> , cercaria of, relation to <i>Cercaria douglasi</i> .....	343
<i>Cotylurus flabelliformis</i> , relation of physid and planorbid snails to life cycle of .....	437
COULSTON, FREDERICK. Thermal deathpoint of the erythrocytic stages of <i>Plasmodium circumflexum</i> .....	365
CRAIG, CHARLES FRANKLIN AND ERNEST CARROLL FAUST. Clinical parasitology (Review) .....	274
<i>Cryptocotyle lingua</i> , correlation between infection of snail hosts and habits of gulls .....	539
CUCKLER, ASHTON C. AND LAWRENCE R. PENNER. <i>Cercaria elongata</i> Brackett, 1940, from a new snail host, <i>Menetus exacuons</i> (Say), in Minnesota .....	187
CULBERTSON, JAMES T. Natural transmission of immunity against <i>Trypanosoma lewisi</i> from vaccinated mother rats to their young .....	75
CULBERTSON, JAMES T. Trypanosomiasis in the Florida cotton rat, <i>Sigmodon hispidus littoralis</i> .....	45
<i>Cystidicola minuta</i> Fujita, new name for .....	542
DAVIS, GORDON E. <i>Ornithodoros parkeri</i> Cooley: Observations on the biology of this tick .....	425
DEEDS, FLOYD AND JOHN O. THOMAS. Studies on phenothiazine. IX. The biliary excretion and anthelmintic action of thionol .....	143
<i>Diectophyma</i> , <i>Diectophyme</i> and <i>Eustrongylus</i> .....	279
Doves and pigeons, American, the slender lice of .....	259
DOWNING, VINCENT F. (see McCoy, Downing and Van Voorhis) .....	53



Eggs, differentiation of <i>Nematodirus filicollis</i> and <i>N. spathiger</i> .....	473
Eggs, <i>Haemonchus contortus</i> , comparison of those in utero with those recovered from feces, and a statistical method for identifying them in mixed infections .....	453
Eggs, <i>Nematodirus</i> , in utero, egg-laying function of a nematode as shown by study of .....	481
<i>Endamoeba histolytica</i> and intestinal nematodes in a Georgia state institution ..	89
<i>Euparyphium melis</i> , studies on life history of .....	35
FARR, MARION M. AND GEORGE W. LUTTERMOSER. Comparative efficiency of zinc sulfate and sugar solutions for the simultaneous flotation of coccidial oöcysts and helminth eggs .....	417
FAUST, ERNEST CARROLL, E. PERRY THOMAS AND JACK JONES. Discovery of human heartworm infection in New Orleans .....	115
FAUST, ERNEST CARROLL (see Craig and Faust) .....	274
FELDMAN, SEYMOUR I. Studies on the morphology and biology of a psilostome fluke .....	525
FINLAY, CARLOS E. Carlos Finlay and yellow fever (Review) .....	367
FISCHTHAL, JACOB H. AND LEONARD M. ALLISON. <i>Acolpenteron ureteroecetes</i> Fischthal and Allison, 1940, a monogenetic trematode from the ureters of the black basses, with a revision of the family Calceostomatidae (Gyrodactyloidea) .....	517
Fishes, California tidepool, and their myxosporidian parasites, on distribution relationships between .....	409
Fluke, blood, from the mallard .....	165
Fluke, <i>Diplorchis scaphiopi</i> , a new polystomatid monogenean, from the spade-foot toad .....	153
Fluke, psilostome, studies on morphology and biology of a .....	525
Fly, stable, occurrence of larvae of, in nymphs of grasshopper .....	90
Frog, green, <i>Rana clamitans</i> , absence of opalinids from the adult .....	513
FUJITA, TSUNENOBU. New names for <i>Metabronema sakvelini</i> Fujita and <i>Cystidicola minuta</i> Fujita .....	542
Gapeworm, <i>Syngamus trachea</i> , in robins and chickens .....	369
GEIGER, J. C. (editor) 1940 year book of public health (Review) .....	275
GOODNIGHT, CLARENCE J. <i>Pterodrilus alvicornus</i> in Virginia .....	468
Gopher, <i>Citellus richardsonii</i> , as experimental host for <i>Trichinella spiralis</i> ...	535
Grasshopper, occurrence of larvae of stable fly in living nymphs of .....	90
Grebe, pied-billed, life history of <i>Echinochasmus donaldsoni</i> n. sp., a trematode from .....	347
GUBERLET, JOHN EARL (In memoriam) .....	188
Guinea pig, <i>Trichostrongylus colubrififormis</i> in .....	363
Gulls, a case of correlation between infection of snail hosts with <i>Cryptocotyle lingua</i> .....	539
<i>Haemonchus contortus</i> eggs: comparison of those in utero with those recovered from feces, and a statistical method of identifying <i>H. contortus</i> eggs in mixed infections .....	453
HAM, COYT (see Young and Ham) .....	71
HARDCASTLE, A. B. <i>Paragonimus</i> in a cat from North Carolina .....	541
HATCH, MELVILLE H. John Earl Guberlet (1887-1940) .....	188
Hawk, <i>Buteo platypterus</i> , a new spiruroid nematode from .....	184
HAZARD, F. O. The absence of opalinids from the adult green frog, <i>Rana clamitans</i> .....	513
Heartworm, human infection in New Orleans .....	115
HEGNER, ROBERT AND REDGINAL HEWITT. Genus name <i>Martinezia</i> changed to <i>Martinezella</i> .....	542

Helminth fauna of a raccoon .....	539
Helminths in partridges and pheasants in Denmark .....	29
Helminths of muskrats in southeast Texas .....	175
Hematozoa from California birds .....	540
Heron, great blue, a new dilepidid cestode from .....	171
HEWITT, REGINAL (see Hegner and Hewitt) .....	542
<i>Hexamita (Otomitus) columbae</i> , occurrence in pigeons in California .....	185
HILL, WILLIAM C. <i>Gryporhynchus tetrorchis</i> , a new dilepidid cestode from the great blue heron .....	171
HINSHAW, W. R. (see McNeil and Hinshaw) .....	185
HOBMAIER, M. Description and extramammalian life of <i>Crenosoma mephitidis</i> n. sp. (Nematoda) in skunks .....	229
HOBMAIER, M. Extramammalian phase of <i>Physaloptera maxillaris</i> Molin, 1860 (Nematoda) .....	233
HOBMAIER, M. Extramammalian phase of <i>Skrjabinigylus chitwoodorum</i> (Nem- atoda) .....	237
HOFF, C. CLAYTON. A case of correlation between infection of snail hosts with <i>Cryptocotyle lingua</i> and the habits of gulls .....	539
HOFFMAN, W. A. (see Maldonado and Hoffman) .....	91
HOPKINS, SEWELL H. New genera and species of the family Monorchidae (Trematoda), with a discussion of the excretory system .....	395
HUSSEY, KATHLEEN L. Partial twinning in a stylet cercaria .....	92
HYMAN, LIBBIE HENRIETTA. The invertebrates: Protozoa through Ctenophora (Review) .....	95
Immunity against <i>Trypanosoma lewisi</i> , natural transmission from vaccinated mother rats to young .....	75
INGRAM, WILLIAM MARCUS. The helminth fauna of a raccoon .....	539
Intestinal nematodes and <i>E. histolytica</i> , incidence in a Georgia state institution .....	89
Intestinal parasites, incidence of, in a mental hospital .....	71
Intestinal protozoa in students .....	90
JANER, JOSÉ L. Miracidial twinning in <i>Schistosoma mansoni</i> .....	93
JONES, JACK (see Faust, Thomas and Jones) .....	115
KATES, K. C. Observations on the viability of eggs of lungworms of swine ...	265
KATZ, JULIUS S. A collection of Ohio ticks and their hosts .....	467
KNUTSON, HERBERT. The occurrence of larvae of the stable fly, <i>Muscina sta-</i> <i>bulans</i> (Zett.) in living nymphs of the grasshopper, <i>Xanthippus corallipes</i> <i>pantherinus</i> (Sc.) .....	90
LANDSBERG, J. W. AND C. F. LISCHER. Anthelmintic studies on the mono ethers of the dihydroxy benzenes .....	357
LARSH, JOHN E., JR. <i>Corallobothrium parvum</i> n. sp., a cestode from the com- mon bullhead, <i>Ameiurus nebulosus</i> Le Sueur .....	221
Leech, <i>Branchellion ravenelii</i> (Girard, 1850), rediscovery and morphology of ..	289
LEIGH, W. HENRY. Variation in a new species of cestode, <i>Railletina (Skrja-</i> <i>binia) variabila</i> , from the prairie chicken in Illinois .....	97
LEONARD, A. BYRON AND ALICE E. LEONARD. The intestinal phase of the re- sistance of rabbits to the larvae of <i>Taenia pisiformis</i> .....	375
LEVIN, ARTHUR J. Recovery of <i>Trichinella spiralis</i> larvae in early stages of infection .....	107
Lice, slender, of American pigeons and doves .....	259
Life cycle of the strigeid trematode, <i>Cotylurus flabelliformis</i> , relation of physid and planorbid snails to the .....	437
Life histories, note on, of genus <i>Paramonostomum</i> (Trematoda: Notocotylidae), with special reference to the excretory vesicle .....	363

Life history of <i>Echinochasmus donaldsoni</i> n. sp., a trematode (Echinostomidae) from the pied-billed grebe .....	347
Life history of <i>Euparyphium melis</i> .....	35
LISCHER, C. F. (see Landsberg and Lischer) .....	357
LUCKER, JOHN T. A redescription of <i>Contracaecum multipapillatum</i> (von Drasche, 1882) (Nematoda: Anisakinae) .....	505
Lungworms of swine, observations on the viability of eggs of .....	265
LUTTERMOSER, GEORGE W. (see Farr and Luttermoser) .....	417
LUTZ, ADOLPHO (In memoriam) .....	469
MCCONNAUGHEY, BAYARD. Two new mesozoa from California, <i>Dicyemenea californica</i> and <i>Dicyemenea brevicephala</i> (Dicyemidae) .....	63
MCCOY, O. R., VINCENT F. DOWNING AND STANLEY N. VAN VOORHIS. The penetration of radioactive phosphorus into encysted <i>Trichinella</i> larvae ....	53
MCCOY, O. R. (see Offutt and McCoy) .....	535
MCIVOR, BARBARA CATHERINE. A new species of <i>Ornithodoros</i> tick from Nevada (Acarina: Ixodoidea) .....	435
McMULLEN, D. B. (see Cort, Olivier and McMullen) .....	123
MCNEIL, E. AND W. R. HINSHAW. The occurrence of <i>Hexamita</i> ( <i>Octomitus</i> ) <i>columbae</i> in pigeons in California .....	185
MADSEN, HOLGER. The occurrence of helminths and coccidia in partridges and pheasants in Denmark .....	29
MALDONADO, JOSÉ F. AND W. A. HOFFMAN. <i>Tamerlanea bragai</i> , a parasite of pigeons in Puerto Rico .....	91
Mallard, a blood fluke, <i>Dendritobilharzia anatinarum</i> , from the .....	165
MANWELL, REGINALD D. Avian toxoplasmosis with invasion of the erythrocytes .....	245
<i>Martinezia</i> genus changed to <i>Martinezella</i> .....	542
Mesozoa, new, from California .....	63
<i>Metabronema salvelini</i> Fujita, new name for .....	542
MEYER, MARVIN C. The rediscovery together with the morphology of the leech, <i>Branchellion ravenelii</i> (Girard, 1850) .....	289
Mice, stomach tube for .....	93
Microtechnique, morphology of some protozoan parasites in relation to .....	1
MIZELLE, JOHN D. Studies on monogenetic trematodes. IV. <i>Anchoradiscus</i> , a new dactylogyrid genus from the bluegill and the stump-knocker sunfish .	159
<i>Moniliformis</i> , specific status of, of Texas rats, and review of genus .....	241
Monkeys, morphology of <i>E. histolytica</i> -like amoebae found in .....	327
Monorchiidae (Trematoda), new genera and species of the family, with a discussion of the excretory system .....	395
MOORMAN, ALBERT E. <i>Balantidium coli</i> and pinworm in a chimpanzee .....	366
Muskrats, helminths of in southeast Texas .....	175
Myiasis, case of urinary .....	465
Myxosporidian parasites, distribution relationships between California tide pool fishes and their .....	409
Nematode, new spiruroid, <i>Habronema americanum</i> , from the broad-winged hawk, <i>Buteo platypterus</i> .....	184
Nematodes from amphibia of Gaspé peninsula .....	59
<i>Nematodirus</i> eggs in utero, egg-laying function of a nematode as shown by study of .....	481
<i>Nematodirus filicollis</i> and <i>N. spathiger</i> , differentiation of eggs of .....	473
New genera, species and varieties (volume 27, 1941)	
<i>Anchoradiscus</i> Mizelle .....	159
<i>Anchoradiscus anchoradiscus</i> Mizelle .....	160
<i>Contracaecum ainterlabium</i> Lucker .....	510
<i>Corallobothrium parvum</i> Larsh .....	221
<i>Crenosoma mephitidis</i> Hobmaier .....	229

<i>Cystidicola chika</i> Fujita .....	542
<i>Dendritobilharzia anatinarum</i> Cheatum .....	166
<i>Dicyememnea brevicephala</i> McConnaughey .....	64
<i>Dicyememnea californica</i> McConnaughey .....	63
<i>Diplomonorchis</i> Hopkins .....	395
<i>Diplomonorchis leiostomi</i> Hopkins .....	395
<i>Diplorchis scaphiopi</i> Rodgers .....	153
<i>Dirofilaria louisianensis</i> Faust, Thomas and Jones .....	121
<i>Echinocasmus donaldsoni</i> Beaver .....	351
<i>Eimeria alabamensis</i> Christensen .....	212
<i>Eimeria subspherica</i> Christensen .....	214
<i>Esthiopterum</i> ( <i>Columbicola</i> ) <i>macrourae</i> Wilson .....	262
<i>Esthiopterum</i> ( <i>Columbicola</i> ) <i>passerinae</i> Wilson .....	259
<i>Genolopa beauforti</i> Hopkins .....	401
<i>Genolopa longovatum</i> Hopkins .....	400
<i>Gryporhynchus tetrorchis</i> Hill .....	171
<i>Habronema americanum</i> Chandler .....	185
<i>Martinezella</i> Hegner and Hewitt .....	542
<i>Metabronema ishii</i> Fujita .....	542
<i>Ornithodoros cooleyi</i> McIvor .....	435
<i>Phagicola lageniformis</i> Chandler .....	176
<i>Postmonorchis</i> Hopkins .....	396
<i>Postmonorchis orthopristis</i> Hopkins .....	397
<i>Raillietina</i> ( <i>Skrjabinia</i> ) <i>variabilis</i> Leigh .....	99
<i>Rhipidocotyle angusticollis</i> Chandler .....	183
<i>Rictularia ondatrae</i> Chandler .....	177
<i>Sphaeromyxa lateralis</i> Noble .....	409
<i>Spironoura rankini</i> Walton .....	59
<i>Sterrhurus texanus</i> Chandler .....	183
<i>Strongyloides ratti ondatrae</i> Chandler .....	180
<i>Trypanosoma sigmodoni</i> Culberston .....	50
NOBLE, ELMER R. On distribution relationships between California tide pool fishes and their myxosporidian (protozoan) parasites .....	409
OFFUTT, EDWARD P., JR. AND O. R. MCCOY. The "gopher," <i>Citellus richardsonii</i> (Sabine), as an experimental host for <i>Trichinella spiralis</i> .....	535
OLIVIER, LOUIS AND W. W. CORT. <i>Cercaria douglasi</i> Cort, 1917 and its relation to the cercaria of <i>Cotylurus flabelliformis</i> (Faust, 1917) .....	343
OLIVIER, LOUIS (see Cort and Olivier) .....	493
OLIVIER, LOUIS (see Cort, Olivier and Brackett) .....	437
OLIVIER, LOUIS (see Cort, Olivier and McMullen) .....	123
Opalinids, absence of, from the adult green frog .....	513
<i>Ornithodoros parkeri</i> Cooley: observations on the biology of this tick .....	425
<i>Ornithodoros</i> tick from Nevada, a new species of .....	435
<i>Otodistomum</i> , the trematode genus in North America .....	253
Oyster drills, polyclad from mantle cavity of .....	541
<i>Paragonimus</i> in a cat from North Carolina .....	541
<i>Paramonostomum</i> Lühe, 1909 (Trematoda: Notocotylidae), note on life histories of, with special reference to excretory vesicle .....	363
Partridges and pheasants in Denmark, helminths and coccidia in .....	29
PENNER, LAWRENCE R. (see Cuckler and Penner) .....	187
Pheasants and partridges in Denmark, helminths and coccidia in .....	29
Phenothiazine, studies on. IX. The biliary excretion and anthelmintic action of thionol .....	143
Phosphorus, radioactive, penetration into encysted <i>Trichinella</i> larvae .....	53
<i>Physa parkeri</i> , larval trematode infection in juveniles and adults of .....	123

<i>Physaloptera maxillaris</i> , extramammalian phase of .....	233
Pigeons and doves, American, the slender lice of .....	259
Pigeons in California, occurrence of <i>Hexamita</i> ( <i>Octomitus</i> ) <i>columbae</i> in .....	185
Pigeons, <i>Tamerlana bragai</i> in, in Puerto Rico .....	91
<i>Plasmodium circumflexum</i> , thermal deathpoint of the erythrocytic stages of .....	365
Polyclad, <i>Hoploplana inquilina thaisana</i> , from mantle cavity of oyster drills .....	541
PRATT, IVAN AND G. D. BARTON. The effects of four species of larval trematodes upon the liver and ovotestis of the snail, <i>Stagnicola emarginata angulata</i> (Sowerby) .....	283
Protozoan parasites, morphology of in relation to microtechnique .....	1
Psilostome fluke, studies on the morphology and biology of a .....	525
<i>Pterodrilus alvicornus</i> in Virginia .....	468
Rabbits and hares, studies on life history of anoplocephaline cestodes of .....	299
Rabbits, intestinal phase of resistance of, to <i>Taenia pisiformis</i> larvae .....	375
Raccoon, helminth fauna of .....	539
Radioactive phosphorus, penetration into encysted <i>Trichinella</i> larvae .....	53
<i>Rana clamitans</i> , green frog, absence of opalinids from the adult .....	513
Rat, <i>Sigmodon hispidus littoralis</i> , Florida cotton, trypanosomiasis in .....	45
Rats of Texas, specific status of <i>Moniliformis</i> of .....	241
REARDON, LUCY V. Incidence of <i>Endamoeba histolytica</i> and intestinal nematodes in a Georgia state institution .....	89
Resistance, intestinal phase of, of rabbits, to <i>Taenia pisiformis</i> larvae .....	375
Respiratory pigments, function of, of turtle parasites .....	81
Reviews:	
BLACKLOCK, D. B. AND T. SOUTHWELL: A guide to human parasitology for medical practitioners .....	274
CHITWOOD, B. G. AND M. B. CHITWOOD: An introduction to nematology ..	273
CRAIG, C. F. AND E. C. FAUST: Clinical parasitology .....	274
FINLAY, CARLOS E. Carlos Finlay and yellow fever .....	367
HYMAN, L. H.: The invertebrates: Protozoa through Ctenophora .....	95
1940 year book of public health .....	275
RIPPLE, RICHARD C. Studies on the gapeworm <i>Syngamus trachea</i> (Montagu, 1811) in robins and chickens .....	369
Robins and chickens, studies on gapeworm, <i>Syngamus trachea</i> in .....	369
RODGERS, L. ORMAND. <i>Diplorchis scaphiopi</i> , a new polystomatid monogenean fluke from the spadefoot toad .....	153
ROTH, HANS. Occurrence of <i>Trichostrongylus colubriformis</i> in the guinea pig ..	363
ROTHSCHILD, MIRIAM. Note on life histories of the genus <i>Paramonostomum</i> Lühe, 1909 (Trematoda: Notocotylidae) with special reference to the excretory vesicle .....	363
RYERSON, DWIGHT L. (see Wohnus and Ryerson) .....	540
SALIS, HERBERT. Studies on the morphology of the <i>E. histolytica</i> -like amoebae found in monkeys .....	327
<i>Schistosoma mansoni</i> , miracidial twinning in .....	93
Sheep, spicule length in <i>Cooperia curticei</i> as a measure of favorable intestinal environment for this intestinal nematode of .....	449
<i>Skrjabingylus chitwoodorum</i> , extramammalian phase of .....	237
Skunks, description and extramammalian life of <i>Crenosoma mephitidis</i> n. sp. from .....	229
Snail hosts, a case of correlation between infection of, with <i>Cryptocotyle lingua</i> , and the habits of gulls .....	539
Snail, <i>Menetus exacuus</i> , a new host of <i>Cercaria elongata</i> .....	187
Snail, <i>Stagnicola emarginata angulata</i> , effects of four species of larval trematodes upon liver and ovotestis of .....	283
Snails, physid and planorbid, relation to life cycle of strigeid trematode, <i>Cotylurus flabelliformis</i> .....	437



SOUTHWELL, T. (see Blacklock and Southwell) .....	274
Spicule length in <i>Cooperia curticei</i> as a measure of favorable intestinal environment for this intestinal nematode of sheep .....	449
SPURLOCK, G. M. A stomach tube for mice .....	93
STABLER, ROBERT M. Intestinal protozoa in 106 parasitology students .....	90
STAUBER, LESLIE A. The polyclad, <i>Hoploplana inquilina thaisana</i> Pearse, 1938, from the mantle cavity of oyster drills .....	541
STILES, CHARLES WARDELL (In memoriam) .....	195
Stomach tube for mice .....	93
Strigeid trematodes, early developmental stages of, in the first intermediate host .....	493
STUNKARD, HORACE W. Adolpho Lutz (1835-1940) .....	469
STUNKARD, HORACE W. Studies on the life history of the anoplocephaline cestodes of hares and rabbits .....	299
Sugar solutions and zinc sulfate, comparative efficiency for simultaneous flotation of coccidial oöcysts and helminth eggs .....	417
Sunfish, stump-knocker, a new dactylogyrid genus from .....	159
Swine, observations on the viability of eggs of lungworms of .....	265
<i>Syngamus trachea</i> in robins and chickens .....	369
<i>Taenia pisiformis</i> larvae, intestinal phase of resistance of rabbits to .....	375
<i>Tamerlanaa bragai</i> , parasite of pigeons in Puerto Rico .....	91
TETLEY, J. H. <i>Haemonchus contortus</i> eggs: comparison of those in utero with those recovered from feces, and a statistical method for identifying <i>H. contortus</i> eggs in mixed infections .....	453
TETLEY, J. H. Spicule length in <i>Cooperia curticei</i> as a measure of favorable intestinal environment for this intestinal nematode of sheep .....	449
TETLEY, J. H. The differentiation of the eggs of the trichostrongylid species <i>Nematodirus filicollis</i> and <i>N. spathiger</i> .....	473
TETLEY, J. H. The egg-laying function of a nematode as shown by study of <i>Nematodirus</i> eggs in utero .....	481
Thermal deathpoint of the erythrocytic stages of <i>Plasmodium circumflexum</i> ..	365
Thionol, biliary excretion and anthelmintic action of. Studies on phenothiazine. IX .....	143
THOMAS, E. PERRY (see Faust, Thomas and Jones) .....	115
THOMAS, JOHN O. (see DeEds and Thomas) .....	143
Ticks and their hosts, a collection of Ohio .....	467
Toad, spadefoot, a new polystomatid monogenean fluke from .....	153
Toxoplasmosis, avian, with invasion of erythrocytes .....	245
TRAVIS, BERNARD V. Examinations of wild animals for the cattle tick <i>Boophilus annulatus microplus</i> (Can.) in Florida .....	465
Trematoda, family Monorchidae, new genera and species of .....	395
Trematode genus <i>Otodistomum</i> in North America .....	253
Trematode, larval, infection in juveniles and adults of <i>Physa parkeri</i> Currier ..	123
Trematode, monogenetic, <i>Acolpenteron ureteroecetes</i> , from ureters of black basses .....	517
Trematodes, larval, effects upon liver and ovotestis of snail .....	283
Trematodes, new, from the bonito, <i>Sarda sarda</i> , in the Gulf of Mexico .....	183
Trematodes, strigeid, early developmental stages of, in the first intermediate host .....	493
Trematodes, studies on monogenetic. IV. <i>Anchoradiscus</i> , a new dactylogyrid genus from the bluegill and the stump-knocker sunfish .....	159
<i>Trichinella</i> larvae, encysted, penetration of radioactive phosphorus into .....	53
<i>Trichinella spiralis</i> larvae, recovery in early stages of infection .....	107
<i>Trichinella spiralis</i> , the "gopher," <i>Citellus richardsonii</i> , as experimental host for ..	535
<i>Trichostrongylus colubrifomis</i> , occurrence in the guinea pig .....	363
<i>Trichuris</i> vs. <i>Trichocephalus</i> .....	279
<i>Trypanosoma lewisi</i> , natural transmission of immunity against, from vaccinated mother rats to young .....	75

Trypanosomiasis in Florida cotton rat, <i>Sigmodon hispidus littoralis</i> .....	45
Turtle parasites, function of respiratory pigments of .....	81
Twinning, miracidial, in <i>Schistosoma mansoni</i> .....	93
Twinning, partial, in stylet cercaria .....	92
VAN CLEAVE, HARLEY J. AND CHARLES M. VAUGHN. The trematode genus	
<i>Otodistomum</i> in North America .....	253
VAN VOORHIS, STANLEY N. (see McCoy, Downing and Van Voorhis) .....	53
VAUGHN, CHARLES M. (see Van Cleave and Vaughn) .....	253
Viability of eggs of lungworms of swine .....	265
WALTON, A. C. Amphibian nematodes from the Gaspé peninsula and vicinity ..	
59	
WENRICH, D. H. Portrait .....	Frontispiece
WENRICH, D. H. The morphology of some protozoan parasites in relation to microtechnique .....	1
WETMORE, PSYCHE W. Blood parasites of birds of the District of Columbia and Patuxent Research Refuge vicinity .....	379
WHARTON, G. W. The function of respiratory pigments of certain turtle para- sites .....	81
WILSON, FRANCIS H. The slender lice of American pigeons and doves with descriptions of two new species .....	259
WOHNUS, J. FREDERICK AND DWIGHT L. RYERSON. Hematozoa from California birds .....	540
WRIGHT, WILLARD H. Charles Wardell Stiles (1867-1941) .....	195
YOUNG, MARTIN D. AND COYT HAM. The incidence of intestinal parasites in a selected group at a mental hospital .....	
71	
Zinc sulfate and sugar solutions, comparative efficiency for simultaneous flota- tion of coccidial oöcysts and helminth eggs .....	417



# INDEX FOR DECEMBER SUPPLEMENT, 1941

(\* refers to abstract number rather than page)

ACKERT, J. E. Natural resistance to helminthic infections .....	45*
ACKERT, J. E. (see Eisenbrandt and Ackert) .....	82*
ACKERT, J. E. (see Frick and Ackert) .....	83*
ACKERT, J. E. (see Reid and Ackert) .....	78*
Acranil, anti-malarial effect of .....	61*
Age resistance, the rôle of duodenal mucus in .....	83*
ALLEN, REX W. (see Farr and Allen) .....	29*
American Society of Parasitologists:	
Constitution and by laws .....	42
Officers .....	39
Program 17th Annual Meeting, Dallas, Texas .....	1
ANDREWS, JOHN S. Some clinical aspects of experimental esophagostomiasis in cattle .....	22*
ANDREWS, JUSTIN and MADGE REYNOLDS <i>Hexamita</i> in the blood of a mouse..	47*
<i>Apophallus brevis</i> , life history of .....	7*
<i>Ascaridia lineata</i> , effects of duodenal mucus of dogs and swine upon the viability of, in vitro .....	82*
<i>Ascaris</i> eggs, hatching of, in vitro .....	21*
Atabrine, effect of, upon experimental cysticercosis in mice .....	79*
AUTHOR INDEX .....	9
AVERY, JOHN L., Reactions in garbage-fed and in grain-fed hogs to intracutaneous injections of various diluents .....	33*
AVERY, JOHN L. (see Spindler and Avery) .....	32*
AVERY, JOHN L. (see Spindler, Avery and Zimmerman) .....	34*
BECKER, ELERY R. Pantothenic acid and <i>Eimeria nieschulzi</i> infection in the white rat .....	55*
BECKMANN, ALBERT J. (see Brackett and Beckmann) .....	70*
BOND, FRANKLYN F. (see McCoy and Bond) .....	41*
BOUGHTON, DONALD C. The effects of sulfaguanidine on experimental bovine coccidiosis .....	19*
BRACKETT, STERLING and ALBERT J. BECKMANN The fate of some species of schistosome cercariae in chick embryos .....	70*
Branchiobdellid, a new species of, from Kentucky .....	75*
BYRD, ELON E. The excretory system of <i>Paragonimus</i> .....	2*
CABLE, R. M. and A. V. HUNNINEN Studies on the life history of <i>Siphodera vinalledwardsii</i> (Linton) (Trematoda: Cryptogonimidae) .....	10*
CABLE, R. M. and A. V. HUNNINEN The systematic position of the genus <i>Deropristis</i> Odhner with respect to a proposed revision of the trematode families Acanthocolpidae and Allocreadiidae .....	14*
CABLE, R. M. (see Hunninen and Cable) .....	8*
CABLE, R. M. (see Hunninen and Cable) .....	9*
Cercariae of digenetic trematodes, development of the excretory system in .....	1*
Cercariae of the common mud-flat snail, <i>Cerithidea californica</i> . I. The pleurolophocercous groups .....	5*
Chagas' disease, reservoir hosts of, in the state of Texas .....	62*
CHANDLER, ASA C. The genus <i>Gnathostoma</i> in North America .....	50*
COATNEY, G. ROBERT and MARTIN D. YOUNG A study of the paroxysms resulting from induced infections of <i>Plasmodium vivax</i> .....	58*
Coccidia, intestinal, of the domestic rabbit, life cycles of four species of .....	57*
Coccidiosis, bovine, effects of sulfaguanidine on .....	19*
Coccidiosis, immunization by the use of x-ray attenuated oöcysts .....	20*

(\* refers to abstract number rather than page)

Coccidiosis of chickens, sulfaguanidine feeding as a control measure for .....	29*
CORT, W. W. AND LOUIS OLIVIER The early developmental stages of <i>Plagiorchis muris</i> (Trematoda: Plagiorchiidae) in its first intermediate host .....	6*
CULBERTSON, JAMES T. AND SYLVIA H. GREENFIELD Effect of atabrine upon experimental cysticercosis in mice .....	79*
Cysticercosis in mice, effect of atabrine upon .....	79*
DELAUNE, ELAINE T. AND ROY L. MAYHEW Further data on the blood picture in stomach worm ( <i>Haemonchus contortus</i> ) infections .....	27*
DENTON, J. FRED Studies on the life history of a dicrocoeliid trematode of the genus <i>Lyperosomum</i> .....	11*
<i>Dicrocoelium dendriticum</i> , occurrence in the United States .....	14*
<i>Dicrocoelium dendriticum</i> , life cycle of .....	12*
DOETSCHMAN, WILLIS H. The occurrence of mites in pinnipeds, including a new species from the California sea-lion, <i>Zalophus californianus</i> .....	37*
DRAKE, TERRENCE J. (see Porter, Swanson and Drake) .....	36*
<i>Duboscqia</i> sp., a microsporidian parasite of <i>Reticulitermes</i> of Maryland .....	69*
<i>Echinocasmus</i> sp., (Trematoda), and a comparison of the excretory system of the cercaria with three related species .....	3*
<i>Eimeria nieschulzi</i> infection in the white rat, pantothenic acid and .....	55*
<i>Eimeria stiedae</i> , transmission of, from the domestic to the cottontail rabbit ....	56*
EISENBRANDT, L. L. AND J. E. ACKERT Effects of duodenal mucus of dogs and swine upon the viability of <i>Ascaridia lineata</i> in vitro .....	82*
<i>Endamoeba histolytica</i> , oxidation-reduction potentials in relation to the cultivation of .....	65*
<i>Endamoeba histolytica</i> from a human case of acute amoebic dysentery, pathogenicity for kittens .....	54*
Esophagostomiasis in cattle, clinical aspects of .....	22*
EVANS, TITUS C. (see Levin and Evans) .....	84*
FARR, MARION M. AND REX W. ALLEN Sulfaguanidine feeding as a control measure for cecal coccidiosis of chickens .....	29*
<i>Fasciola hepatica</i> , the biology and ecology of the snail intermediate host of, in south Texas .....	23*
FEO, L. G. (see Stabler and Feo) .....	68*
Fibroma virus, mechanical transmission of, by certain haematophagous bugs ....	64*
FILLMORE, ROLLIN S. (see Gingrich and Fillmore) .....	61*
FRICK, L. P. AND J. E. ACKERT The rôle of duodenal mucus in age resistance ..	83*
Giardiasis, indicanuria and the vitamin A absorption test in .....	66*
GINGRICH, WENDELL D. AND ROLLIN S. FILLMORE The anti-malarial effect of acranil .....	61*
<i>Gnathostoma</i> in North America .....	50*
GODMAN, GABRIEL C. AND CHARLES H. WILLEY Notes on embryonating eggs of <i>Zygocotyle lunata</i> and on their preparation for cytological study .....	15*
GOODNIGHT, CLARENCE J. A new species of branchiobdellid from Kentucky ....	75*
GRAHAM, GEORGE L. Hatching ascariis eggs in vitro .....	21*
GREENFIELD, SYLVIA H. (see Culbertson and Greenfield) .....	79*
GUTHRIE, JAMES E. (see Harwood and Guthrie) .....	31*
<i>Haemonchus contortus</i> , effects of a low plane of nutrition on .....	26*
<i>Haemonchus contortus</i> in calves, the effect of milk diet on the development of ..	25*
<i>Haemonchus contortus</i> infections, further data on the blood picture .....	27*



(\* refers to abstract number rather than page)

<i>Haemonchus contortus</i> , natural re-infection of calves .....	36*
<i>Haemonchus contortus</i> , notes on the musculature of the male genitalia of .....	28*
<i>Haemonchus</i> larvae, losses of, early after their administration to susceptible sheep .....	24*
Hairworms as parasites of fishes .....	87*
HARRIS, S. T. (see Katsampes, Phillips, McCoord and Harris) .....	66*
HARWOOD, PAUL D. AND JAMES E. GUTHRIE Limited tests of phenothiazine as an anthelmintic in goats .....	31*
HEADLEE, WILLIAM HUGH Observations on the duration of an infection of <i>Hymenolepis nana</i> in man .....	38*
Helminths, frequency of, in their naturally infected hosts .....	73*
HENDERSON, M. E. (see Threlkeld and Henderson) .....	28*
HERMAN, CARLTON M. AND JEANNE YOUNG Blood-cell counts in <i>Plasmodium</i> <i>durae</i> infections .....	60*
HERRICK, C. A. (see Waxler and Herrick) .....	20*
<i>Heterodoxus longitarsus</i> Piaget (Mallophaga: Boopinae), the occurrence of, on dogs in Mississippi .....	63*
<i>Hexamita</i> in the blood of a mouse .....	47*
Hirudinea, North American, further studies on .....	74*
<i>Histomonas</i> from wild pheasants and domestic fowls, some observations on ....	53*
Hookworm, quantitative relationships in immunity to .....	44*
Host-parasite phylogenetic complex: the criteria and their application to the opalinid-anuran complex .....	52*
HSÜ, H. F. AND S. Y. LI The frequency of helminths in their naturally infected hosts .....	73*
HUFF, CLAY G. Saurian malaria .....	59*
HUNNINEN, A. V. AND R. M. CABLE The life history of <i>Podocotyle atomon</i> (Rudolphi) (Trematoda: Opecoelidae) .....	8*
HUNNINEN, A. V. AND R. M. GABLE Studies on the life history of <i>Lecithaster confusus</i> Odhner (Trematoda: Hemiuridae) .....	9*
HUNNINEN, A. V. (see Cable and Hunninen) .....	10*
HUNNINEN, A. V. (see Cable and Hunninen) .....	14*
HUNTER, GEORGE W., III. Studies on host-parasite reactions. VI. An hypothesis to account for pigmented metacercarial cysts in fish .....	71*
HUNTER, GEORGE W., III, AND WANDA S. HUNTER Studies on host-parasite reactions. V. The integumentary type of strigeid cyst .....	17*
HUNTER, WANDA SANBORN Studies on cercariae of the common mud-flat snail, <i>Cerithidea californica</i> . I. The pleurolophocercous groups .....	5*
HUNTER, WANDA S. (see Hunter and Hunter) .....	17*
HUSSEY, KATHLEEN L. Development of the excretory system in cercariae of digenetic trematodes .....	1*
<i>Hymenolepis nana</i> , the duration of infection in man .....	38*
<i>Hymenolepis nana</i> var. <i>fraterna</i> , demonstration of naturally acquired, passive immunity to .....	85*
Immunity to hookworm, quantitative relationships in .....	44*
Immunity to <i>Hymenolepis nana</i> var. <i>fraterna</i> , demonstration of naturally acquired, passive immunity .....	85*
Immunity to the stomach worm ( <i>Haemonchus contortus</i> ), effects of a low plane of nutrition (cottonseed hulls) on .....	26*
Immunity to <i>Trichinella spiralis</i> , attempted passive transfer to rats and mice ..	41*
Immunization against coccidiosis by the use of x-ray attenuated oöcysts .....	20*
JACOBS, LEON Oxidation-reduction potentials in relation to the cultivation of <i>Endamoeba histolytica</i> .....	65*

(\* refers to abstract number rather than page)

JANKIEWICZ, HARRY A. Transmission of the liver coccidium, <i>Eimeria stiedae</i> , from the domestic to the cottontail rabbit .....	56*
KATES, KENNETH C. Experimental infection of pigs with the swine thorn-headed worm, <i>Macracanthorhynchus hirudinaceus</i> .....	35*
KATES, KENNETH C. (see Spindler and Kates) .....	30*
KATSAMPES, C. P., W. A. PHILLIPS, A. B. MCCOORD AND S. T. HARRIS Indicanuria and the vitamin A absorption test in giardiasis .....	66*
KESSEL, JOHN F. (see Rutherford and Kessel) .....	57*
KINCHELOW, WILLIAM D. (see Price and Kinchelov) .....	12*
KUDO, RICHARD R. Notes on a microsporidian parasite, <i>Duboscqia</i> sp., of <i>Reticulitermes</i> of Maryland .....	69*
LARSH, JOHN E., JR. Demonstration of naturally acquired, passive immunity to <i>Hymenolepis nana</i> var. <i>fraterna</i> .....	85*
LASKEY, MARION (see Rothman and Laskey) .....	67*
<i>Lecithaster confusus</i> Odhner, studies on the life history .....	9*
<i>Leidynema appendiculatum</i> , a nematode parasitic in cockroaches, an addition to the life history .....	77*
LEVIN, ARTHUR J. AND TITUS C. EVANS Intestinal resistance to a second infection of <i>Trichinella spiralis</i> .....	84*
LI, S. L. (see Hsü and Li) .....	73*
Life cycle of <i>Diocotophyma renale</i> , the giant kidney worm of mammals .....	80*
Life history and development of <i>Macracanthorhynchus ingens</i> and <i>Mediorhynchus grandis</i> .....	76*
Life history of a microcoelid trematode of the genus <i>Lyperosomum</i> , studies on ..	11*
Life history of <i>Apophallus brevis</i> Ransom, 1920 .....	7*
Life history of <i>Lecithaster confusus</i> Odhner, studies on .....	9*
Life history of <i>Leidynema appendiculatum</i> (Leidy, 1850) Chitwood, 1932, a nematode parasitic in cockroaches, an addition to .....	77*
Life history of <i>Podocotyle atomon</i> (Rudolphi) (Trematoda: Opecoelidae) ....	8*
Life history of <i>Siphodera vinaldwarsi</i> (Linton), studies on .....	10*
LUTERMOSER, GEORGE W. The possibility of chemical control of snail intermediate hosts of <i>Schistosoma mansoni</i> in Venezuela .....	81*
LYON, MARCUS W. AND JOHN D. MIZELLE A case of intestinal myiasis .....	46*
<i>Lyperosomum</i> , studies on the life history of a species of .....	11*
<i>Macracanthorhynchus hirudinaceus</i> , experimental infection of pigs .....	35*
<i>Macracanthorhynchus ingens</i> , life history and development .....	76*
Malaria, saurian .....	59*
MANTER, H. W. Observations on the geographical distribution of digenetic trematodes of marine fishes .....	72*
MANTER, H. W. Two unusual trematodes .....	48*
MAYHEW, ROY L. Studies on bovine gastro-intestinal parasites: VII. The effects of a low plane of nutrition (cottonseed hulls) on immunity to the stomach worm ( <i>Haemonchus contortus</i> ) .....	26*
MAYHEW, ROY L. (see Delaune and Mayhew) .....	27*
MCCOORD, A. B. (see Katsampes, Phillips, McCoord and Harris) .....	66*
MCCOY, O. R. AND FRANKLYN F. BOND Attempted passive transfer to rats and mice of immunity to <i>Trichinella spiralis</i> .....	41*
MCINTOSH, ALLEN The designation of type specimens in describing new species .....	4*
<i>Mediorhynchus grandis</i> , life history and development .....	76*
MESERVE, F. G. <i>Phyllodistomum coatneyi</i> n. sp., a trematode from the urinary bladder of <i>Ambystoma maculatum</i> (Shaw) .....	13*
Metacercarial cysts in fish, an hypothesis to account for pigment in .....	71*

(\* refers to abstract number rather than page)

MEYER, MARVIN C. Further studies on the North American Hirudinea .....	74*
Microsporidian parasite, <i>Duboscqia</i> sp., of <i>Reticulitermes</i> of Maryland, notes on	69*
MILLER, MAX J. The life history of <i>Apophallus brevis</i> Ransom, 1920 .....	7*
Mites, the occurrence of, in pinnipeds, including a new species from the California sea-lion, <i>Zalophus californianus</i> .....	37*
MIZELLE, JOHN D. (see Lyon and Mizelle) .....	46*
MOHR, JOHN LUTHER The host-parasite phylogenetic complex: The criteria and their application to the opalinid-anuran complex .....	52*
MOORE, DONALD V. Life history and development of <i>Macracanthorhynchus ingens</i> and <i>Mediorhynchus grandis</i> (Acanthocephala) .....	76*
MORGAN, BANNER BILL The incidence of <i>Trichomonas foetus</i> in Wisconsin cattle .....	18*
Myiasis, intestinal, a case of .....	46*
Natural resistance to helminthic infections .....	45*
Nematode from a water scavenger beetle, a new species of .....	51*
Nematode parasites of cattle, duration of infectivity on Florida pasture with observations on resistance of calves to natural reinfection with <i>Haemonchus contortus</i> .....	36*
NIGRELLI, ROSS F. Hairworms as parasites of fishes .....	87*
NIGRELLI, ROSS F. Parasites of the green turtle, <i>Chelonia mydas</i> (L.), with special reference to the rediscovery of trematodes described by Looss from this host species .....	16*
NOLF, L. O. AND HERMAN ZAIMAN The effect of host age on the number of <i>Trichinella spiralis</i> recovered from rats during the early period of infection	39*
Officers, American Society of Parasitologists .....	39
OFFUTT, EDWARD P., JR. The effects of immune serum upon the larvae of <i>Trichinella spiralis</i> in vitro .....	40*
OLIVIER, LOUIS (see Cort and Olivier) .....	6*
OLSEN, O. WILFORD The biology and ecology of the snail, <i>Stagnicola bulimoides techella</i> (Hald.), intermediate host of <i>Fasciola hepatica</i> Linn., in south Texas .....	23*
OLSEN, YELENA PAVLINOVA (see Taliaferro and Olsen) .....	42*
OTTO, G. F. Quantitative relationships in immunity to hookworm .....	44*
Oxidation-reduction potentials in relation to the cultivation of <i>Endamoeba histolytica</i> .....	65*
PACKCHANIAN, A. Reservoir hosts of Chagas' disease in the state of Texas ..	62*
Pantothenic acid and <i>Eimeria nieschulzi</i> infection in the white rat .....	55*
<i>Paragonimus</i> , the excretory system of .....	2*
Parasites of the green turtle, <i>Chelonia mydas</i> (L.), with special reference to the rediscovery of trematodes described by Looss from this host species .....	16*
Phenothiazine as an anthelmintic in goats .....	31*
Phenothiazine, effect of, upon egg production and viability of the eggs of swine lungworms .....	30*
PHILIP, CORNELIUS B. Mechanical transmission of Shope rabbit fibroma by certain haematophagous bugs .....	64*
PHILLIPS, W. A. (see Katsampes, Phillips, McCoord and Harris) .....	66*
PORTER, DALE A. The effect of milk diet on the development of <i>Haemonchus contortus</i> in calves .....	25*
PORTER, DALE A., LEONARD E. SWANSON AND TERRENCE J. DRAKE The duration of infectivity of nematode parasites of cattle on Florida pasture with observations on resistance of calves to natural re-infection with <i>Haemonchus contortus</i> .....	36*
PRICE, EMMETT W. AND WILLIAM D. KINCHELOW The occurrence of <i>Dicrocoelium dendriticum</i> in the United States .....	12*

(\* refers to abstract number rather than page)

<i>Phyllodistomum coatneyi</i> n. sp., a trematode from the urinary bladder of <i>Ambystoma maculatum</i> (Shaw) .....	13*
<i>Plagiorchis muris</i> , early developmental stages of, in its first intermediate host ..	6*
<i>Plasmodium durae</i> infections, blood-cell counts in .....	60*
<i>Plasmodium vivax</i> , a study of the paroxysms resulting from induced infections	58*
<i>Podocotyle atomon</i> (Rudolphi), life history of .....	8*
<i>Porrocaecum</i> (Nematoda), two species of, from the robin .....	49*
Precipitate formation around trichina larvae in sera from trichina-infected, and trichina-free hogs .....	34*
Protozoan infection, incidence of, amongst the personnel of a large general hospital with special regard to incidence according to race and occupation ....	67*
Reactions in garbage-fed and in grain-fed hogs to intracutaneous injections of various diluents .....	33*
REARDON, LUCY V. (see Rees and Reardon) .....	54*
REES, CHARLES W. AND LUCY V. REARDON The pathogenicity for kittens of <i>Endamoeba histolytica</i> from a human case of acute amoebic dysentery .....	54*
REID, W. M. AND J. E. ACKERT Removal of chicken tapeworms by host starvation and some effects of such treatment on tapeworm metabolism .....	78*
Reservoir host of Chagas' disease in the state of Texas .....	62*
REYNOLDS, MADGE (see Andrews and Reynolds) .....	47*
ROTHMAN, MAURICE M. AND MARION LASKEY Incidence of protozoan infection amongst the personnel of a large general hospital with special regard to incidence according to race and occupation .....	67*
RUTHERFORD, ROBERT L. AND JOHN F. KESSEL Life cycles of four species of intestinal coccidia of the domestic rabbit .....	57*
<i>Schistosoma mansoni</i> in Venezuela, the possibility of chemical control of the snail intermediate hosts of .....	81*
<i>Schistosoma cercariae</i> , the fate of, in chick embryos .....	70*
<i>Siphodera vinaldwadsi</i> (Linton), studies on the life history .....	10*
Skin and precipitin tests for the diagnosis of trichina infections in grain-fed and in garbage-fed hogs .....	32*
SPINDLER, L. A. AND JOHN L. AVERY Skin and precipitin tests for the diagnosis of trichina infections in grain-fed and in garbage-fed hogs .....	32*
SPINDLER, L. A. AND KENNETH C. KATES Effect of a single dose of phenothiazine upon egg production and viability of the eggs of swine lungworms	30*
SPINDLER, L. A., JOHN L. AVERY AND HARRY E. ZIMMERMAN, JR. Precipitate formation around trichina larvae in sera from trichina-infected and trichina-free hogs .....	34*
STABLER, ROBERT M. AND L. G. FEO Survival time of intravaginally implanted <i>Trichomonas hominis</i> .....	68*
STOLL, NORMAN R. AND J. H. TETLEY Losses of <i>Haemonchus</i> larvae early after their administration to susceptible sheep .....	24*
Strigeid cyst, integumentary type of .....	17*
Sulfaguanidine, effects of, on experimental bovine coccidiosis .....	19*
Sulfaguanidine feeding as a control measure for cecal coccidiosis of chickens ...	29*
SWANSON, LEONARD E. (see Porter, Swanson and Drake) .....	36*
TALIAFERRO, LUCY GRAVES (see Taliaferro and Taliaferro) .....	43*
TALIAFERRO, WILLIAM H. AND LUCY GRAVES TALIAFERRO The trypanocidal action of sheep serum on <i>Trypanosoma duttoni</i> .....	43*
TALIAFERRO, WILLIAM H. AND YELENA PAVLINOVA OLSEN The protective action of normal sheep serum against infections of <i>Trypanosoma duttoni</i> in the mouse .....	42*

(\* refers to abstract number rather than page)

Tapeworms, removal of, from chickens by host starvation and some effects of such treatment on tapeworm metabolism .....	78*
Testosterone, effects of, on nematodes .....	86*
TETLEY, J. H. (see Stoll and Tetley) .....	24*
THOMAS, LYEEL J. Effects of testosterone on nematodes .....	86*
THRELKELD, WILLIAM LOGAN AND M. E. HENDERSON Notes on the musculature of the male genitalia of <i>Haemonchus contortus</i> .....	28*
TODD, A. C. An addition to the life history of <i>Leidynema appendiculatum</i> (Leidy, 1850) Chitwood, 1932, a nematode parasitic in cockroaches .....	77*
TODD, A. C. Demonstration of a new parasitic nematode from a water scavenger beetle .....	51*
TOWNSEND, ELSIE W. Preliminary report on <i>Echinochasmus</i> sp. (Trematoda), and a comparison of the excretory system of the cercaria with three related species .....	3*
Transmission, mechanical, of Shope rabbit fibroma by certain haematophagous bugs .....	64*
Trematodes of marine fishes, observations on the geographical distribution of ..	72*
Trematodes, two unusual species .....	48*
Trichina infections in hogs, skin and precipitin tests for the diagnosis of .....	32*
Trichina larvae, precipitate formation in sera from trichina-infected and trichina-free hogs .....	34*
<i>Trichinella spiralis</i> , attempted passive transfer of immunity to rats and mice ...	41*
<i>Trichinella spiralis</i> , intestinal resistance to a second infection of .....	84*
<i>Trichinella spiralis</i> , the effect of host age on the number recovered from rats during the early period of infection .....	39*
<i>Trichinella spiralis</i> , the effects of immune serum upon the larvae in vitro .....	40*
<i>Trichomonas foetus</i> , incidence of, in Wisconsin cattle .....	18*
<i>Trichomonas hominis</i> , survival time of, implanted intravaginally .....	68*
<i>Trypanosoma duttoni</i> , the protective action of normal sheep serum against infections of, in the mouse .....	42*
<i>Trypanosoma duttoni</i> , the trypanocidal action of sheep serum on .....	43*
Type specimens, the designation of, in describing new species .....	4*
WARD, JAMES W. The occurrence of <i>Heterodoxus longitarsus</i> Piaget (Mallophaga: Boopinae) on dogs in Mississippi .....	63*
WAXLER, S. H. AND C. A. HERRICK Immunization against coccidiosis by the use of x-ray attenuated oöcysts .....	20*
WEBSTER, J. D. Two species of <i>Porrocaecum</i> (Nematoda) from the robin ...	49*
WENRICH, D. H. Some observations on <i>Histomonas</i> from wild pheasants and domestic fowls .....	53*
WILLEY, CHARLES H. (see Godman and Willey) .....	15*
WOODHEAD, ARTHUR E. The life cycle of <i>Diectophyma renale</i> , the giant kidney worm of mammals .....	80*
YOUNG, JEANNE (see Herman and Young) .....	60*
YOUNG, MARTIN D. (see Coatney and Young) .....	58*
ZAIMAN, HERMAN (see Nolf and Zaiman) .....	39*
ZIMMERMAN, HARRY E., JR. (see Spindler, Avery and Zimmerman) .....	34*
<i>Zygocotyle lunata</i> , notes on embryonating eggs and on their preparation for cytological study .....	15*